

# **Characterisation of the pharmacological actions in humans of multiple vasoactive enzyme inhibitors with therapeutic potential in heart failure**

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## **1.1 Abstract**

### **Introduction**

The work described in this thesis looks particularly but not exclusively at two recently developed molecules which have dual enzyme inhibitor activity. Omapatrilat, a molecule which inhibits both angiotensin converting enzyme (ACE) and neutral endopeptidase (NEP), and SLV 306 (active metabolite KC12615) a compound with both NEP and endothelin converting enzyme (ECE) inhibiting properties.

Neurohumoral activation characterises the complex chronic heart failure syndrome. Clearly there is value in antagonizing neurohumoral systems likely to have detrimental effects in patients with heart failure, while simultaneously augmenting potentially desirable neurohumoral mediators. However enzyme inhibitors which act on multiple vasoactive mediators with complex interactions have unpredictable effects.

Omapatrilat has received much attention following demonstration of a powerful hypotensive effect but a higher than expected incidence of angioedema in patients with hypertension or heart failure. GW660511X is another dual ACE/NEP inhibitor at an earlier stage in development. The pharmacodynamic profile of neither the ACE inhibitor activity nor the NEP inhibitor activity of GW 660511X has been fully described in humans. SLV 306 is the first orally available molecule of its kind and its NEP and ECE inhibitory properties have not previously been demonstrated in humans either *in vitro* or *in vivo*.

The intention of this thesis is further characterisation of these molecules and their therapeutic potential while utilising their inhibitory properties in further investigation of the human neurohumoral system. I specifically consider the possible mediators of the impressive hypotensive effects of these molecules and of the unexpected and potentially life threatening side effects associated with them. Having demonstrated the complex neurohumoral substrate of these molecules I go on to report, for the first time in heart failure patients, the benefits of a more specific approach to neurohumoral manipulation using a recently developed renin inhibitor, aliskiren. Aliskiren has been shown to offer haemodynamic benefit in patients with hypertension but has not previously been given to patients with chronic heart failure.

## **Methods**

- 1) Small resistance arteries from patients (n=89) with coronary artery disease but normal left ventricular function were studied using wire myography. The vasopressor response to various neurohormones in the presence of omapatrilat, KC12615 (the active metabolite of SLV 306) and other vasoactive enzyme inhibitors is reported.
- 2) Following pilot studies of the pressor response to intravenous infusion of big ET-1 (n=6) and pharmacokinetic modeling of orally dosed SLV 306 in healthy volunteers (n=29), the effect of 3 doses of SLV 306 and placebo given at four separate visits one week apart, on the pressor and neurohumoral response to intravenous infusion of big endothelin-1 in healthy volunteers (n=15) is compared.
- 3) The effect of 2 oral doses of GW660511X and a single dose of the ACE inhibitor ramipril, given on three separate visits one week apart, on the pressor and neurohumoral response to an intravenous infusion of angiotensin I in healthy volunteers (n=16) is compared.
- 4) Finally, the neurohumoral and blood pressure response to aliskiren an orally active, long acting renin inhibitor is compared with placebo for one week and the ACE inhibitor ramipril for 6 weeks, in patients with left ventricular systolic dysfunction (n=27).

## **Results**

- 1) In patients with coronary artery disease but normal left ventricular systolic dysfunction; the vasodilator response to bradykinin was augmented by omapatrilat, KC 12615, phosphoramidon (NEP/ECE inhibitor), captopril (ACE inhibitor), and thiorphan (NEP inhibitor). Of note the augmentation is no greater with omapatrilat than captopril and in arteries taken from patients prescribed ACE inhibitor, KC 12615 does not augment the response. The vasodilator response to adrenomedullin was augmented by omapatrilat, KC 12615 and phosphoramidon. The vasoconstrictor response to angiotensin I was inhibited by omapatrilat and captopril and the vasoconstrictor response to endothelin-1 was inhibited by KC 12615 and phosphoramidon.
- 2) In healthy volunteers, SLV 306 caused a dose dependent attenuation of the hypertensive and reflex bradycardia response to big ET-1. There was also a dose

dependent increase in ANP, big ET-1 and the ratio big ET-1: ET-1 but no increase in ET-1 following big ET-1 infusion.

3) In healthy volunteers, there was no notable change in blood pressure (pre angiotensin I infusion) and no significant inhibition of pressor response to angiotensin I following administration of GW660511X. Ramipril 10mg was associated with a reduction in blood pressure (pre angiotensin I infusion) and inhibition of the response to angiotensin I. There was significantly greater reduction in ACE activity with ramipril than GW660511X. GW660511X but not ramipril led to a dose dependent increase in plasma ANP concentration.

4) In patients with chronic heart failure, aliskiren suppressed plasma renin activity and reduced plasma angiotensin II. Ramipril in comparison caused an increase in renin activity and no change in angiotensin II. There were no significant changes in blood pressure with either treatment.

## **Conclusion**

I have demonstrated the ACE and NEP inhibitory properties of omapatrilat and for the first time in humans, the ECE and NEP inhibitory properties of SLV 306, in vitro in patients with coronary artery disease but normal left ventricular dysfunction. I found no additional augmentation of bradykinin by omapatrilat or SLV 306 over and above that offered by ACE inhibition but significant augmentation by both dual inhibitors of adrenomedullin. This contradicts the suggestion that bradykinin has a role in the incidence of angioedema offers adrenomedullin as an alternative mediator. Adrenomedullin augmentation may also contribute significantly to the hypotensive effects of these molecules.

I have demonstrated for the first time in humans the ECE and NEP inhibitory properties of SLV 306 in vivo.

GW660511X is shown to inhibit NEP but to a much lesser extent ACE. Of note the comparison made is with full dose of a powerful pure ACE inhibitor. Any inhibition of ACE activity in contrast to the study of pure NEP inhibitors is consistent with the belief that dual inhibition offers additional benefit.

Finally I have demonstrated for the first time in patients with chronic heart failure the renin inhibitor activity of aliskiren, confirming attenuation of the renin angiotensin aldosterone pathway consistently from its origin and in contrast the rise in renin activity seen with ACE inhibitors.

## **1.2 Declaration**

I declare that this thesis has been composed by myself and is a record of work performed by myself unless otherwise stated. It has not been previously submitted for any other degree. The work described in this thesis was carried out under the supervision of Professor JJV McMurray in the Department of Medicine and Therapeutics at the Western Infirmary in Glasgow.

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Alison Seed

September 2007

### **1.3 Acknowledgements**

I would like to offer sincere thanks to my supervisor, Professor John McMurray, for his advice, encouragement and careful supervision throughout my period of research.

I am grateful to Ms. Fiona Johnston who taught and assisted me in the technique of wire myography (chapters 3, 4 and 5), she performed a proportion of these experiments, and Dr. Chris Hillier who advised me with regard to statistical analysis and interpretation of the wire myography data.

Solvay Pharmaceuticals (CJ Van Houtenlaan 36. NL-1381 CP Weesp. The Netherlands) provided financial support and assistance with statistical analysis of data for my work with SLV 306 (Chapters 5 and 7). They also performed all the pharmacokinetic modelling of SLV 306 and its metabolite KC 12615 reported in chapter 7. GlaxoSmithKline (Greenford Road, Greenford. Middlesex UB6 0HE) provided financial support and assistance with statistical analysis of data for my work with GW660511X (chapter 8). They performed all pharmacokinetic modelling required as part of the study reported in chapter 8. Speedel pharmaceuticals (Speedel Pharma AG, Hirschgässlein 11, CH – 4051 Basel, Switzerland) provided financial support and assistance with statistical analysis of data for my work with aliskiren (chapter 9).

Neurohumoral analyses in each of my studies were performed by specialist biochemists. Dr. J. Morton, Dr. A. Davenport and Dr. J. Nussberger are credited individually for their welcome contribution in this regard where appropriate.

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## List of Publications

A. Seed, R. Gardner, J. McMurray, C. Hillier, D. Murdoch, R. McFadyen, A. Bobillier, J. Mann, T. McDonagh.

Neurohumoral effects of the new orally active renin inhibitor, aliskiren, in chronic heart failure.

*European Journal of Heart Failure*: 9 (11); 1120-7. October 2007

A. Seed, M.P. Love, J.J. McMurray.

Clinical experience with endothelin receptor antagonists in chronic heart failure.

*Heart Failure Review* 6 (4); 317-23. December 2001

A. Seed, J. McMurray.

Current approaches to treating heart failure.

*Prescriber* 12; 75-89. May 2001

A. Seed, J. McMurray.

Management of heart failure.

*Chapter, Horizons in medicine, Royal College of Physicians.*

C. Berry, J. McMurray, L. Blue, A. Seed.

Multidisciplinary strategies to improve the management of heart failure patients in the community.

*Cardiology news*. 4; 6-11. April-May 2001

## Presentations to learned societies

A. Seed, C.Hillier, C.Berry, F.Johnston, M. Petrie, J. McMurray.

Evidence that a novel orally active NEP/ECE inhibitor blocks the vasoconstriction response to big endothelin in human resistance arteries.

- American College of Cardiology. March 2001.
- Seventh international conference on endothelin, September 2001.
- Medical Research Society. February 2002.



C.Berry, C.Hillier, A. Seed, J.Irvine, M.Petrie, JJV. McMurray.

The effects of omapatrilat on the vasomotor response to neurohumoral peptides in resistance arteries from patients with coronary heart disease.

- American College of Cardiology. March 2001.

A. Seed, C.Hillier, C.Berry, F.Johnston, A.Brown, M.Petrie, JJV. McMurray.

An interaction between ACE inhibition and NEP/ECE inhibition – effects on vasodilator peptides.

- Seventh international conference on endothelin. September 2001.
- Medical Research Society. February 2002.

A. Seed, R.Kuc, A.Davenport, M.Ashby, C.Hillier, P.Passier, H.Essers, J. McMurray.

First demonstration in humans of systemic NEP and ECE inhibition using a new, orally active, dual metalloprotease inhibitor, SLV 306.

- Seventh international conference on endothelin. September 2001.
- Finalist Research prize RCPS (Glasgow). Nov 2001(oral)
- Medical Research Society. February 2002.
- British Cardiac Society. May 2002 (oral)

A. Seed, R. Gardner, J. McMurray, C. Hillier, D. Murdoch, R. McFadyen, A. Bobillier, J. Mann, T. McDonagh.

First Experience with an orally active renin inhibitor (aliskiren) in patients with chronic heart failure.

- British Cardiac Society. May 2003 (oral)

RE Kuc, MJ Ashby, A Seed, P Passier, H Essers, J McMurray, AP Davenport.

The ECE /NEP inhibitor SLV 306 (Daglutril), inhibits systemic endogenous conversion of infused big endothelin–1 in human volunteers.

- British Pharmacological Society, Winter meeting 2005. 3(4) abstract 126P

## **Letters**

A. Seed, J. McMurray.

Acute endothelin A receptor blockade in heart failure.

*Circulation 101(25); 2922-7. June 2000*

## List of Abbreviations

<b>ACE</b>	Angiotensin converting enzyme
<b>ADM</b>	Adrenomedullin
<b>ANP</b>	Atrial natriuretic peptide
<b>AIRE</b>	The Acute Infarction Ramipril Efficacy study
<b>ANOVA</b>	Analysis of variance
<b>AIIRA</b>	Angiotensin II receptor antagonist
<b>BMI</b>	Body mass index
<b>BNP</b>	Brain natriuretic peptide
<b>CCRC</b>	Cumulative concentration response curve
<b>cGMP</b>	Cyclic guanosine monophosphate
<b>CGRP</b>	Calcitonin gene related peptide
<b>CHF</b>	Chronic heart failure
<b>CIBIS II</b>	Cardiac Insufficiency Bisoprolol Study
<b>CONSENSUS</b>	Co-operative North Scandanavian Enalapril Survival Study
<b>CNP</b>	C-natriuretic peptide
<b>DNP</b>	Dendroaspis natriuretic peptide
<b>DR-1</b>	Dose-response ratio-1
<b>EARTH</b>	Endothelin Receptor Antagonist Trial in Heart failure
<b>ECE</b>	Endothelin converting enzyme
<b>EDHF</b>	Endothelin derived hyperpolarizing factor
<b>EDRF</b>	Endothelin derived relaxing factor
<b>ELITE</b>	Evaluation of Losartan in the Elderly study
<b>ESC</b>	European Cardiac Society
<b>ET</b>	Endothelin
<b>EUROPA</b>	EUROpean trial On reduction of cardiac events with Perindopril in stable coronary Artery disease Investigators
<b>FDA</b>	Food and Drug Administration
<b>GISSI</b>	Gruppo Italiano per lo Studio della Streptochinasi nell Infarto Miocardiso
<b>HOPE</b>	Heart Outcomes Prevention Evaluation study
<b>ISIS-2</b>	Second International Study of Infaret Survival

<b>LDL</b>	Low density lipoprotein
<b>LVDT</b>	Linear Variable Differential Transformer
<b>MAP</b>	Mean arterial pressure
<b>MERIT-HF</b>	Metoprolol CR XL Randomised Intervention Trial in Congestive Heart Failure
<b>NE</b>	Norepinephrine
<b>NEP</b>	Neutral endopeptidase
<b>NO</b>	Nitric oxide
<b>NOS</b>	Nitric oxide synthase
<b>NYHA</b>	New York Heart Association
<b>OCTAVE</b>	Omapatrilat Cardiovascular Treatment vs. Enalapril trial
<b>OPERA</b>	Omapatrilat in Persons with Enhanced Risk of Atherosclerotic Events
<b>OVERTURE</b>	Omapatrilat Versus Enalapril Randomized Trial of Utility in Reducing Events
<b>PSS</b>	Physiological saline solution
<b>RAAS</b>	Renin angiotensin aldosterone system
<b>REACH</b>	Research on Endothelin Antagonism in Chronic Heart failure trial.
<b>SOLVD</b>	Study of Left Ventricular Dysfunction
<b>SRA</b>	Subcutaneous resistance arteries
<b>VIP</b>	Vasoactive intestinal peptide

# **1 INTRODUCTION**

## **1.1 Heart Failure**

### **1.1.1 A DEFINITION**

Before discussing the neurohormones involved in the pathophysiology of heart failure and the complex vasoactive enzyme inhibitors offering new potential for treatment, it is important to define the syndrome to which I am referring. Heart failure is difficult to define since it encompasses a number of differing pathologies and different symptom profiles.

Heart failure, by strict definition, is an inability of the heart to deliver sufficient blood and therefore oxygen to the tissues of the body.

It may be an acute problem, for example as the result of arrhythmia or recent myocardial infarct. It may be as a result of chronic deterioration in cardiac function, for example as a result of valvular dysfunction, hypertension, alcohol excess or previous myocardial infarct.

Many texts discuss left and right heart failure as two distinct diagnoses. This is misleading. Although it is true that different pathologies may affect the left and right ventricles to a varying degree, it is not helpful to discuss the two as entirely separate conditions. Traditionally right heart failure refers to patients with salt and water retention causing peripheral oedema, ascites and elevated jugular venous pressure. Left heart failure refers to those with acute pulmonary oedema and hypotension. However, it is understood that salt and water retention is often the result of systemic neurohormonal activation, a consequence in many cases of left sided systolic dysfunction. In a significant number of patients it is a combination of left and right ventricular dysfunction that results in the symptoms and signs associated with heart failure.

It might be suggested that a helpful definition of heart failure relies on a constellation of symptoms and signs or a syndrome. Defining heart failure as a group of symptoms however, excludes patients who despite impairment in myocardial contractility do not report symptoms. Perhaps a group to which treatment offers considerable long term benefit. Also nearly half of all patients with the symptoms and signs of heart failure are

found to have a normal left ventricular ejection fraction (Sanderson 2007). The prognosis of the patient said to have a normal ejection fraction and the therapeutic approach to them has been shown to be different to that of the patient with systolic dysfunction heart failure (Vasan et al 1995). The terms used to describe this condition are numerous. Examples include ‘diastolic dysfunction’ and ‘preserved left ventricular systolic function heart failure’. Both terms are misleading. The term ‘diastolic dysfunction’ is often not based on objective evidence of diastolic impairment since traditional non invasive measurements require refinement. ‘Preserved left ventricular systolic function’ often relies on traditional assessments of ejection fraction which are not sensitive. Many of these patients clearly do not have normal systolic function. Indeed it is artificial to consider the two phases of the cardiac cycle as independent of one another. Ventricular filling or diastolic function is clearly dependent in part on effective ventricular emptying and left ventricular ‘suction’. Ventricular hypertrophy and fibrosis traditionally associated with impaired filling quite clearly affect contractility as well as relaxation of the myocardium.

Most recently, studies of patients with symptoms of heart failure but apparently preserved left ventricular systolic function have suggested that there is primarily a problem with arterial compliance resulting in abnormal ventriculo – arterial coupling. (Burkhoff et al 2003, Balmain et al 2007). Endothelial function, key to maintaining normal vascular tone may play a significant role in this syndrome. Endothelial dysfunction or abnormal vasomotor control has been well documented in patients with impaired left ventricular systolic function, indeed many of the treatments, particularly involving neurohumoral modulation, target this pathophysiological process.

Symptoms alone clearly cannot be relied on as a sensitive or specific indicator of left ventricular function.

Since the advent of two-dimensional echocardiography many research groups have used objective measurement of left ventricular systolic dysfunction either with or without symptoms to define the population to be studied. Indeed, the development in recent years of newer echocardiographic techniques, such as tissue Doppler imaging, has enabled an even more accurate assessment of ventricular systolic function. Measurement of long axis left ventricular function demonstrates reduced function in

many patients with signs and symptoms of heart failure but ‘normal’ ejection fraction when compared to an asymptomatic age matched population (Yip et al 2002).

In 2001 the European Cardiac Society (ESC) chose to define heart failure in terms of both symptom profile *and* objective evidence of left ventricular systolic dysfunction; ‘typically breathlessness or fatigue, either at rest or during exercise, or ankle swelling; and objective evidence of cardiac dysfunction at rest (usually on 2D echocardiography) (Remme and Swedberg 2001).

While clearly still excluding some patient groups the guideline has at least defined a relatively homogenous group of patients in whom useful research can be performed. This encourages large, well designed, randomised, controlled trials and epidemiological studies in populations that are well defined. The results of these studies can be appropriately applied to large patient groups.

Brain natriuretic peptide (BNP) measurement perhaps offers an additional tool for diagnosis in the future. It has an exceptionally high negative predictive value (Cowie et al 1997) The negative predictive value of a normal electrocardiogram to exclude left ventricular systolic dysfunction is over 90%.(Gillespie et al 1997, Rihal et al 1995) However, many physicians are not sufficiently practised at interpreting electrocardiograms to confidently pass one as normal and several workers have suggested that estimation of plasma BNP in those who present with suspected left ventricular systolic dysfunction could be a more objective measure used to select patients for echocardiography (Yamamoto et al 2000, Cowie et al 1997). The current ESC guidelines suggest estimation of plasma BNP levels as an aid in the diagnosis of heart failure when it is available (Remme and Swedberg 2001)

In addition to being clear about the definition of heart failure, it is important clinically that the diagnosis is never considered in isolation but as the consequence of a primary underlying condition. Along with the initiation of appropriate heart failure therapy, identification of the primary diagnosis also allows initiation of the appropriate treatment for that condition. For example the symptoms and signs of heart failure due to critical aortic stenosis may be similar to those seen in poor left ventricular systolic function secondary to previous coronary artery disease but clearly the pathophysiology and treatment of these conditions is very different.

For the purpose of this thesis when I refer to heart failure I talk specifically about symptomatic, echocardiographically confirmed left ventricular systolic dysfunction.

### 1.1.2 EPIDEMIOLOGY

Heart failure is recognised as a major cause of morbidity and mortality across the developed world. It is undoubtedly an increasing problem both in terms of incidence and prevalence.

Perhaps the accessibility of two dimensional echocardiography and therefore improvement in diagnostic rates contributes to an increase in reported incidence. Perhaps improved data collection makes a difference, with improved consensus on the definition of heart failure playing a part. However with official statistics attributing only a small proportion of heart failure deaths to heart failure, tending instead to record the underlying aetiology, there is little doubt that the true contribution of heart failure to overall mortality continues to be significantly underestimated.

The incidence of heart failure is reported at between 1 and 16/1000 persons. This figure increases with age and is significantly higher in men than in women (Ho, Pinsky et al 1993, Cowie et al 1999).

The prevalence of heart failure is more widely reported. Stewart et al estimated that in 1995 there were almost one million patients in the United Kingdom with symptomatic heart failure requiring treatment (Stewart et al 2002). An estimate, by the same group, of the burden on the National Health Service in 2000 suggested that heart failure was responsible for almost 2% of its total annual expenditure at that time. This rises to as much as 4% if hospital admissions in which heart failure is not the primary but the secondary or tertiary diagnosis are included. Only ten years previously it was suggested that heart failure was responsible for 1.2% of the National Health Service total expenditure. Clearly despite improvements in treatment the socioeconomic burden of heart failure continues to rise. There are a number of factors which may contribute to this.

Firstly, the prevalence of heart failure is increasing because of, rather than despite, improvements in treatment. Advances in our understanding of and therefore our treatment of cardiovascular disease, particularly the use of thrombolysis in myocardial

infarction (GISSI 1986, ISIS-2 1988) and the use of angiotensin converting enzyme (ACE) inhibitors in heart failure (Garg and Yusuf 1995) have improved the longevity of these patients. Secondly, major advances in the treatments available for heart failure, while improving mortality and morbidity also mean an increase in the total cost per patient (Stewart et al 2002). Finally and perhaps most significantly, our population is an aging one and the prevalence of heart failure increases considerably with age. Population screening reported in the Framingham study shows a prevalence of 8/1000 in those less than 60 years of age, rising to 23/1000 in those less than 70 years and as much as 91/1000 in those over the age of 80 years (Ho, Pinsky et al 1993). Studies of patients visiting general practitioners with symptoms suggestive of heart failure, identified as those being prescribed diuretics, report an overall prevalence of 3-15 cases per 1000, with 28-80 cases per 1000 in those over 65 years of age (McMurray and Stewart 2000). The population prevalence of left ventricular systolic dysfunction as determined by echocardiography, irrespective of the presence of symptoms, is reported as 29 per 1000 overall rising to 64 per 1000 in men over the age of 65 years (McDonagh et al 1997).

### 1.1.3 PROGNOSIS

As well as being relatively common, heart failure is a chronic, disabling condition with a very poor prognosis.

It is worth highlighting that the standard method of classifying clinical severity of heart failure is the New York Heart Association (NYHA) Classification. (See table 1.1 below). NYHA class is related to prognosis in patients with heart failure.

However, the correlation between clinical features and echocardiographic assessment of left ventricular systolic function is poor. Some individuals with moderate to severe left ventricular systolic dysfunction on imaging exhibit only mild symptoms or are even asymptomatic. Indeed, it has been suggested that approximately half of all individuals with left ventricular systolic dysfunction are asymptomatic (Remme & Swedberg 2001). Conversely patients may exhibit severe symptoms of heart failure clinically with only mild left ventricular systolic dysfunction on imaging. Until the introduction of ACE inhibitors for the treatment of chronic heart failure the mortality of the condition had not improved in over 40 years (Ho, Anderson et al 1993). Even following their introduction



**Table 1.1** New York Heart Association classification of heart failure.

NYHA Class:	Symptoms:
I	No limitation: ordinary physical exercise does not cause undue fatigue, dyspnoea or palpitations.
II	Slight limitation of physical activity: comfortable at rest but less than ordinary activity results in fatigue.
III	Marked limitation of physical activity: comfortable at rest but less than ordinary activity results in symptoms.
IV	Unable to carry out any physical activity without discomfort: symptoms of heart failure are present even at rest with increased discomfort with any physical activity.

the five year survival following diagnosis is as low as 25%, a poorer outcome than that seen in many forms of cancer (Stewart et al 2001). Almost half of patients can expect to die within a year of diagnosis, with the elderly having the worst prognosis (McMurray and Stewart 2000). Women tend to live longer than men explaining a similar prevalence between the sexes despite a higher incidence in men (MacIntyre et al 2000). Improved blood pressure control has also had a more positive effect on the incidence of heart failure in women than in men. In men the improvement in blood pressure control is offset by an increased survival post myocardial infarction with heart failure. Although the mortality figures are alarming, the true overall mortality attributable to heart failure is likely in fact to be underestimated. Official records of cause of death often attribute the death to the primary condition for example coronary artery disease or valve disease rather than to the true terminal condition – heart failure.

#### 1.1.4 AETIOLOGY

Prior to the availability of anti hypertensive drugs, the commonest cause of heart failure in the developed world was hypertension (Nicholls 1996). Indeed, during the same era, heart failure was reported as the commonest cause of death in patients with

hypertension. More recently outcomes of hypertension have been less accurately reported. Metanalyses often concentrate on stroke and myocardial infarction since the rate of heart failure as a consequence of hypertension is more difficult to assess in a standardised way.

In the modern era it is more often stated that coronary artery disease is the commonest cause of heart failure (Gheorghiade and Bonow 1998, Fox et al 1999, Cowie et al 1999). A review of clinical trials and registers, between 1991 and 1999 attributed 54-71% of cases to ischaemia and no more than 20% to hypertension. Of some concern is the relative frequency of cases of heart failure described as of unknown aetiology (Cowie et al 1999).

Some would suggest that myocardial ischaemia or infarct are simply the mechanism linking hypertension with heart failure (Nicholls 1996) Ischaemia being recognised more often now with improved access to diagnostic coronary angiography. Unfortunately the evidence provided is somewhat unreliable. Hypertension is largely asymptomatic until complications occur and with the onset of heart failure arterial pressure almost invariably falls. Hypertension becomes a hidden aetiology. There is consistent evidence however that effective treatment of hypertension reduces the incidence of heart failure by up to 50% (Moser and Herbert 1996). So why, as treatment of hypertension undoubtedly improves, is the incidence and prevalence of heart failure increasing? Firstly, inadequate control of hypertension is important, perhaps delaying rather than preventing heart failure. Secondly, as suggested previously, improved survival from myocardial infarction is an important contributor.

#### 1.1.5 PATHOPHYSIOLOGY

Chronic heart failure (CHF) is a progressive pathological process. It develops initially as a physiological response to reduced cardiac output. Relative arterial under filling is detected by systemic baroreceptors and results in heightened sympathetic nervous system activation. This and renal hypoperfusion as a result of the reduction in cardiac output triggers activation of the renin angiotensin aldosterone system (RAAS). The consequences being vasoconstriction, sodium and water retention and vascular remodelling.

To be more specific, in the face of a disturbance in myocardial contractility the heart depends on three adaptive mechanisms to maintain adequate circulation. 1) an increased preload reflected in an elevation in end diastolic volume maintaining the Frank Starling mechanism and therefore cardiac output, 2) activation of neurohumoral systems, augmenting myocardial contractility and maintenance of arterial pressure and therefore vital organ perfusion and 3) myocardial remodelling possibly with ventricular dilatation in order to increase the mass of contractile tissue. The first two will occur rapidly, the latter perhaps over weeks or months.

Unfortunately the capacity of each to maintain systemic vascular resistance and cardiac output is limited and when chronically maintained becomes maladaptive. The resulting reduction in vascular compliance increases not only cardiac preload but also afterload, to which the failing heart is particularly sensitive.

Vasoconstriction, as a result of these physiological mechanisms combined with endothelial dysfunction (considered in greater detail in a later chapter), is the process responsible for much of the multi-system pathology associated with CHF.

- Vasoconstriction of skeletal muscle vasculature results in impaired vasodilatory capacity during exercise, contributing to the classic symptoms of fatigue and exercise intolerance (Zelis et al 1974).
- Increased pulmonary vascular resistance compromises right ventricular function resulting in secondary fluid retention and in the long term pulmonary hypertension. Patients are often short of breath even at rest.
- Renal vasoconstriction occurs early (Vanhoutte 1983) and results in further reduction in renal perfusion and therefore RAAS activation and increased sodium and water retention.

The standard treatments for CHF, hypertension and ischaemic heart disease, act by systemic means to reduce vascular tone amongst other actions. However, locally acting factors and specifically endothelin-1, have been shown to be the most potent vasoconstrictors (Yanagisawa et al 1988). The production, action and degradation of these vasoactive peptides have therefore become exciting new therapeutic targets.

Though, at least initially, a primary mechanical and haemodynamic problem, it is recognised that neurohumoral activation characterises the more complex CHF syndrome (Cohn et al 1981, Francis 1998). Although initially a physiological response to reduced cardiac output, chronic activation of certain neurohumoral systems, e.g. The RAAS, seems to lead to undesirable renal effects and to functional and structural modifications of the heart and peripheral vasculature that result in hypertrophy and fibrosis (figure 1.1). In addition to the detrimental haemodynamic effects described above. This pathological, sustained, neurohumoral over activity is believed to be a major cause of progression of CHF i.e. an explanation for the steady haemodynamic and symptomatic decline that characterises the CHF syndrome.

Neurohormonal data from the SOLVD study show that there is a progressive increase in plasma concentrations of renin, noradrenalin, aldosterone and atrial natriuretic peptide (ANP) from normal to asymptomatic left ventricular dysfunction and from asymptomatic left ventricular dysfunction to overt heart failure (Francis et al 1990). An increase in plasma endothelin-1 has also been reported in severe heart failure (NYHA III-IV) with an inverse correlation between plasma endothelin-1 and ejection fraction (Wei et al 1994). Neurohormonal activation has also been reported in post infarction left ventricular systolic function (Rouleau et al 1993) even in the asymptomatic. It is said that neurohormonal activation correlates directly with severity of heart failure, more specifically ejection fraction than severity of symptoms (Benedict et al 1994).

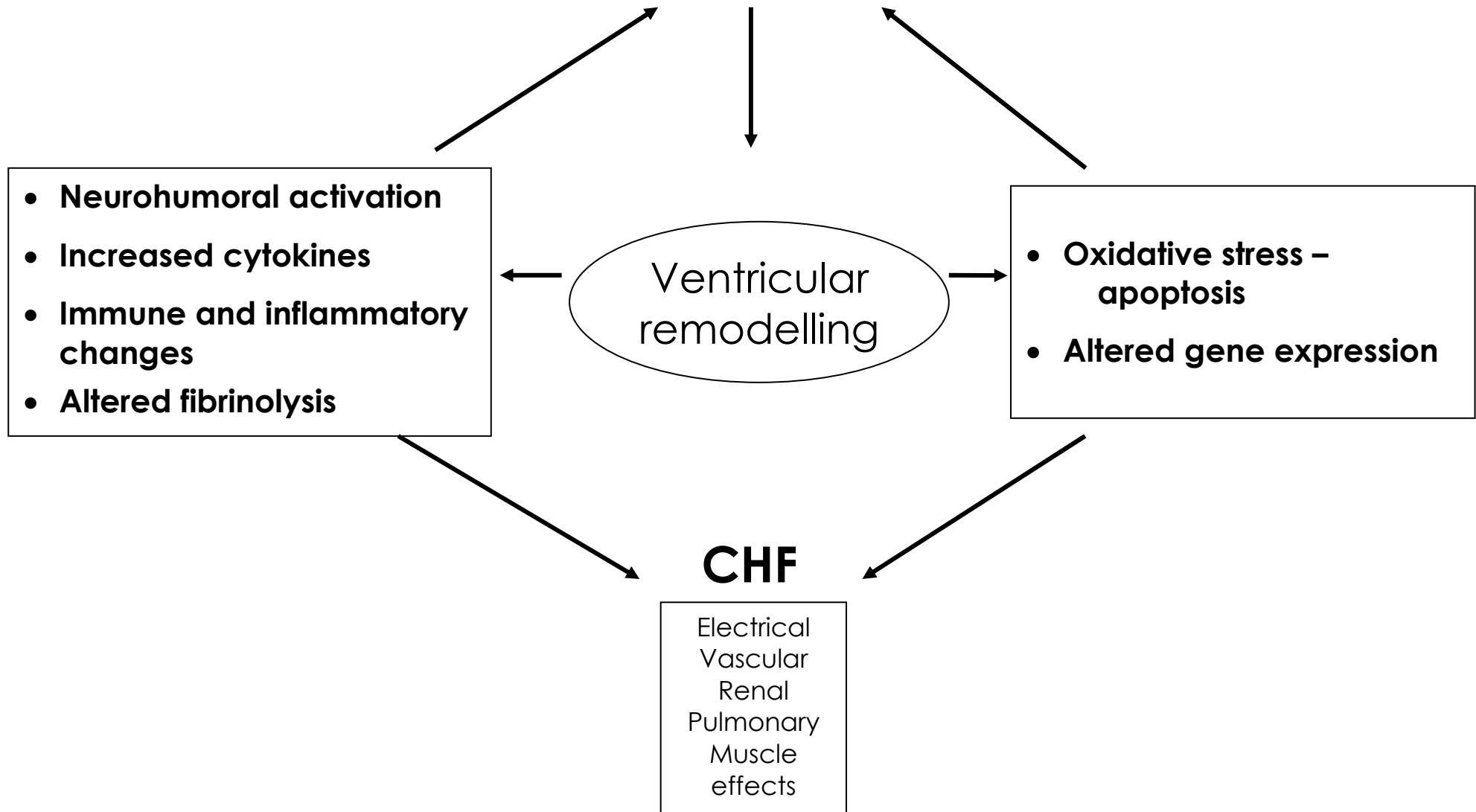
Neurohormonal activation has also been recognised as a predictor of cardiovascular mortality. In moderate and severe heart failure the highest mortality occurs in patients with the highest plasma levels of noradrenaline, ANP and renin activity (Swedberg et al 1990) Plasma ANP, renin activity and aldosterone are independent predictors of mortality and occurrence of acute heart failure following myocardial infarction (Rouleau et al 1994). Increased plasma concentrations of endothelin-1 and its precursor big endothelin-1 have also been associated with a poor prognosis. In fact it has been shown that the endothelins are more powerful predictors of mortality than many haemodynamic or other hormone studies (Tsutamoto et al 1995, Pacher et al 1996, Pousset et al 1997).

Neurohormonal activation is often considered in terms of a balance between vasodilating or natriuretic and vasoconstrictive or antinatriuretic forces. In addition to

**Figure 1.1**

The neurohumoral model of heart failure

**Injury to myocytes and extra cellular matrix**



improving our understanding of the pathophysiology of CHF, this neurohumoral paradigm has given us a framework for understanding therapeutic interventions in CHF. Initially, we recognised the value of antagonising neurohumoral systems likely to have detrimental actions in CHF; more recently it has become clear that to achieve this while simultaneously augmenting potentially desirable neurohumoral mediators may be the optimum strategy.

ACE inhibitors (CONSENSUS 1987, SOLVD 1991, 1992), beta blockers (CIBIS II 1999, Merit HF 1999) and spironolactone (Pitt et al 1999) inhibit undesirable neurohumoral pathways and are associated with significant improvements in the morbidity and mortality of heart failure. Diuretics therapy is a well established and effective treatment associated with symptom reduction in heart failure (Anand et al 1990). However, they have been associated with raised plasma renin activity and aldosterone secretion. They may well stimulate these undesirable neurohumoral axes. The use of these agents should almost certainly be restricted to the management of discrete periods of fluid retention and lowered to the lowest effective dose once the acute episode is controlled (Bayliss et al 1987). We continue to look for more effective treatment strategies and most recently attention has turned to the possibility of stimulating beneficial neurohumoral systems such as the natriuretic peptides (Chen and Burnett 1999) in addition to inhibiting those which are detrimental.

In summary, the natural history of patients with CHF, one of steady haemodynamic and symptomatic decline, is in part the result of a maladaptive physiological response to reduced myocardial contractility, mediated by the activation of multiple neural and humoral vasoconstrictor reflexes aimed at preserving cardiac output and vital organ perfusion.

Over time this process results in increased systemic vascular resistance, further impairment of left ventricular ejection fraction, and vasoconstriction in the renal, muscle and pulmonary vasculature causing predictable clinical and pathophysiological deterioration. Despite remarkable advances in our understanding of CHF as a pathological process, it continues to be a leading cause of death in many countries of the world. It is not only a cause of great mortality but is also associated with significant morbidity. Quality of life due to reduced exercise tolerance and fatigue is often very poor in patients with chronic heart failure, while frequent, lengthy hospital stays, often

with intensive acute management, result in huge financial burden. The focus of scientific attention has been on neurohumoral systems with potentially undesirable effects in CHF and there is an excellent evidence base enabling firm recommendations to be made for the treatment of CHF.

The work described in this thesis looks particularly but not exclusively at two recently developed molecules which have dual enzyme inhibitor activity. Omapatrilat, a molecule which inhibits both ACE and neutral endopeptidase (NEP), and SLV 306 (active metabolite KC12615) a compound with both NEP and endothelin converting enzyme (ECE) inhibiting properties. It considers the therapeutic potential of these agents and also makes use of them as tools to further our understanding of the mechanisms involved. Particularly considering the unpredictable effects of enzyme inhibitors which act on multiple vasoactive mediators with potentially complex interactions.

## **1.2 NEUROHORMONES – targets for therapy**

Neurohormones are chemical substances, produced by specialized neurons, which act either locally or systemically to change the structure or function of target organs. These local and systemic mediators coordinate a complex system.

The sympathetic nervous system stimulates the RAAS system which acts centrally and peripherally to further increase sympathetic activity and increase ET-1 and aldosterone production. The endothelium is a major source of paracrine mediators such as nitric oxide (NO) and endothelin (ET) -1. The natriuretic peptide system including ANP and BNP, of myocardial origin, and c-type natriuretic peptide (CNP), of endothelial origin counteract the RAAS and ET systems. The interaction between the components of this complex system is not fully understood. I will go on to discuss these neurohormones and others in more detail

For some time it has been recognised that there is value in antagonising neurohumoral systems likely to have detrimental actions, more recently we have come to realise that the optimum strategy might be to achieve this while simultaneously augmenting potentially desirable neurohumoral mediators. (figure 1.2)

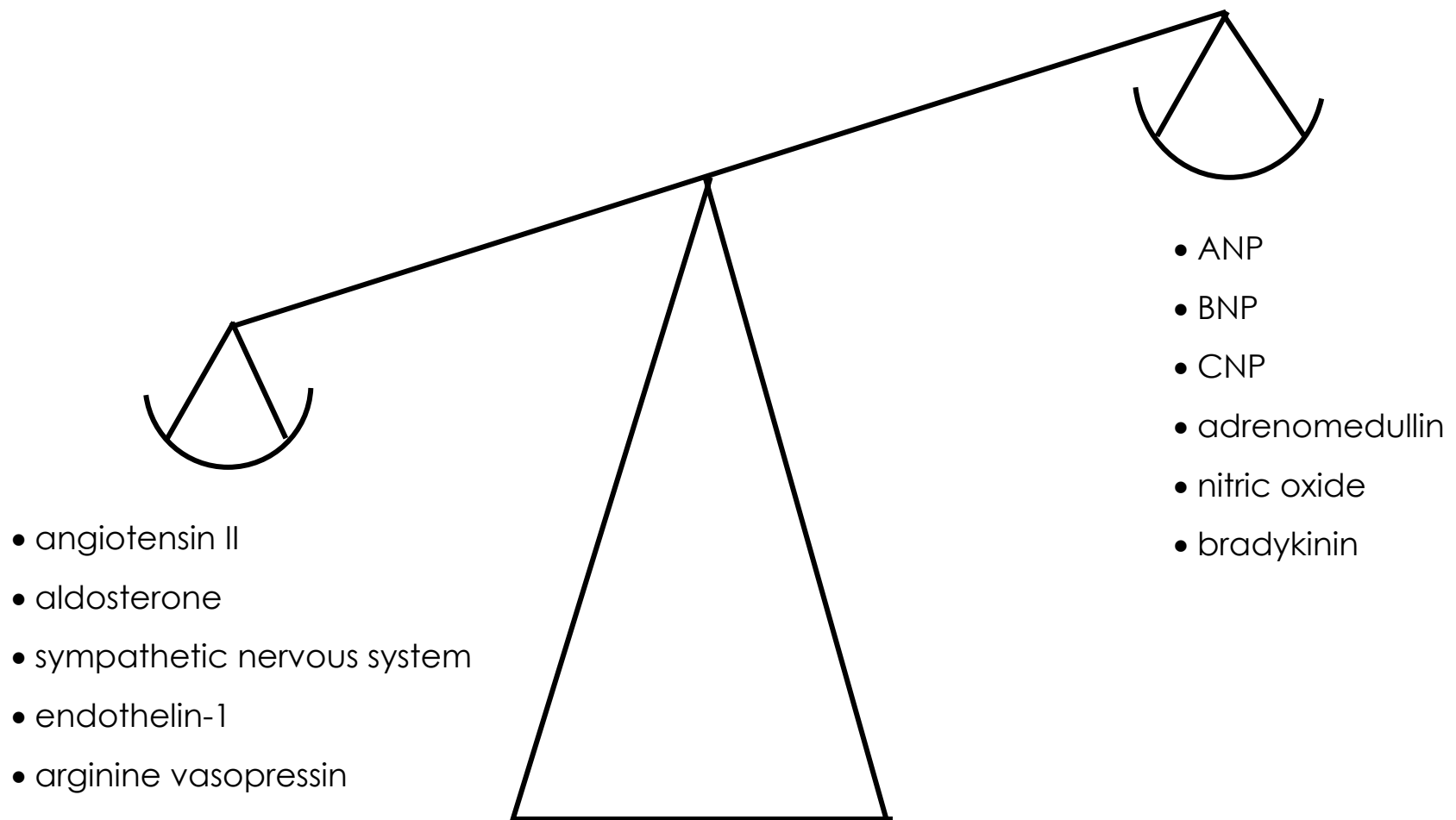
### **1.2.1 THE RENIN ANGIOTENSIN ALDOSTERONE SYSTEM**

Renin initiates the RAAS cascade. It is produced by the juxtaglomerular cells of the kidney in response to stimulation from the sympathetic nervous system or in response to reduced renal perfusion, during periods registered as volume deficiency. It controls the first rate limiting step of the RAAS system. It cleaves amino acids from a peptide produced in the liver, angiotensinogen to release angiotensin I. Angiotensin I is converted to angiotensin II via the action of ACE and chymase. ACE is an identical molecule to kininase II, the enzyme responsible for the metabolism of bradykinin (Yang et al 1970), it is found in abundance in the endothelium as well as in cardiac myocytes, fibroblasts and vascular smooth muscle cells. Chymase is a protease found in interstitial cells. It is thought that greater than 80% of the generation of angiotensin is via the ACE pathway (Zisman et al 1995). Studies in the failing human heart indicate that the ACE gene (Studer et al 1994) and protein expression and enzyme activity (Zisman et al 1998) are increased but chymase gene expression is not (Studer et al 1994).



**Figure 1.2**

Neurohumoral modulation in heart failure. The best “neurohumoral approach” to heart failure therapy may be to simultaneously block undesirable neurohumoral pathways (eg the RAAS) while augmenting potentially beneficial axes (eg the natriuretic peptides)



Angiotensin II is the primary vasoactive hormone of the RAAS system. It is formed systemically as well as locally in cardiac, pulmonary and vascular tissue. The AT<sub>1</sub> receptor is believed to mediate all the conventionally understood actions of angiotensin II. The function of the AT<sub>2</sub> and other putative receptors in humans is not known (Cao et al 1999). Angiotensin II has both direct and indirect roles in the development of hypertension and in the establishment of the haemodynamic syndrome which characterises heart failure. It increases vascular resistance, a primary haemodynamic mechanism. The effects of angiotensin II on aldosterone results in an increase in reabsorption of sodium and water by the kidney, in turn thought to increase blood pressure and further increase cardiac afterload. Angiotensin II also accentuates sympathetic nervous system activity and Angiotensin II and aldosterone have both been found to increase fibrosis and inhibit fibrinolysis (Cody et al 1997). In addition, angiotensin II inhibits renin release, thus providing a negative feedback to the system. The degradation of angiotensin II is thought, in part at least, to be under the control of NEP (Richards et al 1993).

Aldosterone is a mineralocorticoid produced by the outer layer or glomerulosa of the adrenal cortex. It has two important functions 1) regulation of extracellular fluid volume by its effect on sodium retention and 2) potassium metabolism. It acts predominantly on the distal convoluted tubule and collecting duct of the kidney where it promotes reabsorption of sodium. Potassium then diffuses into the lumen of the tubules because of the change in electromagnetic gradient produced by active reabsorption of the positively charge sodium ion. Hydrogen ions may also be more freely excreted.

Aldosterone release is not only regulated by the renin angiotensin system, to which it is also linked in a negative feedback loop. Potassium regulates aldosterone secretion independent of the RAAS; an elevated potassium concentration increasing aldosterone secretion and vice versa. Elevated aldosterone levels have many detrimental effects in heart failure.

In the failing heart it causes changes in blood pressure and cardiac afterload via its effects on salt and water and potassium metabolism. It also has an effect on cardiac fibrosis and remodelling (Cody et al 1997, Sun et al 2002) and the inflammatory response associated with microvascular disease. It causes an increase in sympathetic nervous system activity, evidenced by attenuation of the baroreflex and impaired heart

rate variability, which together with myocardial fibrosis reduces the arrhythmogenic threshold and increases the risk of sudden cardiac death. Increased plasma aldosterone levels in hypertensive patients are associated with reduced systemic arterial compliance (Blacher et al 1997) and plasma aldosterone levels closely correlate with left ventricular mass in this population (Schunkert et al 1997).

As part of this thesis I further characterise a peptide which has been shown to inhibit ACE and NEP, Omapatrilat (Trippodo et al 1998). This molecule may overcome the problems faced by pure NEP inhibitors which were disappointing in the treatment of hypertension in randomized trials (Richards et al 1993) by offering the additional benefit of inhibition of the RAAS system. I also study a similar molecule in an earlier stage of development GW660511X. Finally I study a recent development in the setting of chronic heart failure, an orally active renin inhibitor which promises to inhibit the RAAS system at its origin, Aliskiren.

### 1.2.2 THE ENDOTHELIN SYSTEM

Three structurally similar endothelin isopeptides have been isolated, ET-1, ET-2, and ET-3. It is ET-1 that has been measured as the predominant isoform in human plasma. It is a powerful vasoconstrictor; demonstrated to have ten times the vasoconstrictor potency of angiotensin II, a mitogenic and anti-natriuretic peptide which is produced in increased quantities in CHF (Yanagisawa et al 1988). ET-2 has not been measured in human plasma but can be found in endothelial cells and has been shown to have a similar effect on vascular tone. ET-3 has less potent vasoconstrictor activity and its physiological role remains less clearly understood.

ET-1 stimulates the production of cytokines and growth factors (Hofman et al 1998, Matsuura et al 1998). It interacts with blood cells stimulating neutrophil adhesion (Lopez Farre et al 1993) and platelet aggregation (Knofler et al 1995). In summary it promotes vasoconstriction, cell growth, cell adhesion and thrombosis; it is believed therefore not only to contribute the progression of CHF but also to the development and progression of atherosclerosis and the development of hypertension.

ET-1 is generated from a precursor peptide big ET-1, by ECE. ECE has not been found in human plasma suggesting that it is not systemically active but is produced and acts only locally in specific systems. ECE shares structural and perhaps functional

similarities with NEP (Turner and Tanzawa 1997). It has been suggested that ECE is not selective for big ET-1 but also hydrolyzes peptides such as bradykinin (Hoang and Turner 1997) and substance P (Johnson et al 1999).

ET-1 is predominantly released from the endothelium, abluminally toward the vascular smooth muscle again suggesting a paracrine role (Wagner et al 1992). It is also produced by other cells involved in vascular disease such as leukocytes (Sessa et al 1991) and macrophages (Ehrenreich et al 1990). ET-1 production is regulated by physical factors such as shear stress (MacArthur et al 1994), pulsatile stretch (Malek and Izumo 1992) and pH (Wesson et al 1998). Hypoxia is a strong stimulus for ET-1 synthesis perhaps important in ischaemia (Rakugi et al 1990). Synthesis is also stimulated by cardiovascular risk factors such as elevated levels of LDL cholesterol and glucose, obesity and ageing (Boulanger et al 1992, Yamauchi et al 1990, Barton et al 2000)

Infusion of thiorphan into the human forearm, leading to local NEP inhibition, causes vasoconstriction at least in part reversed by a co-administration of an ET receptor antagonist (Plumpton et al 1995, Haynes and Webb 1994, Love et al 1996, Ferro et al 1998). Candoxatril, another NEP inhibitor, increases plasma ET-1 concentrations (Ando et al 1995, McDowell et al 1997). Both these observations suggest that NEP may contribute to degradation of ET-1 (Russell et al 1996). This may in part explain the rather disappointing antihypertensive effects of NEP inhibitors in vivo (Richards et al 1993).

As part of this thesis I further characterise a peptide which has been shown to inhibit NEP and ECE, SLV 306 (Meil et al 1998). This molecule may offer more effective treatment for patients with hypertension or CHF.

### 1.2.3 NATRIURETIC PEPTIDES

The natriuretic peptides have potent natriuretic (de Bold et al 1981), vasodilator (Currie et al 1983, Webb DJ et al 1998), and antimitogenic (Itoh et al 1990) properties.

ANP is produced primarily by the cardiac atria. Several hormones and neurotransmitters, such as ET, vasopressin and catecholamines directly stimulate the secretion of ANP. Increased atrial wall tension, reflecting increased intravascular

volume is the dominant stimulus for its release (Ogawa et al 1991) both in patients with heart failure and healthy individuals. Plasma concentrations of ANP are augmented in patients with increased intravascular volume, such as those with CHF. This includes patients with a raised left atrial pressure and normal left ventricle for example patients with atrial arrhythmia.

BNP, originally identified in porcine brain tissue (Minamino et al 1988) is in fact predominantly produced by the cardiac ventricles (La Pointe et al 1996). Pro-BNP (108 amino acids) is stored in myocardial cells and cleaved into BNP (32 amino acids) and the inactive N-terminal BNP when it is secreted. Patients with CHF have high plasma concentrations of BNP (Cowie 2000) while concentrations are extremely low in normal individuals. Increasing BNP levels correlate well with worsening NYHA class (Kuster et al 2002) offering a more objective measure. Elevated BNP can be used to identify patients with previously undiagnosed heart failure, a recognised threshold being 18pg/ml (McDonagh et al 1998) and higher levels taken to reflect more severe heart failure. 100pg/ml is recognised as evidence for decompensation (McCulloch et al 2003). At acute presentation an elevated BNP greater than 80-100pg/ml is highly accurate at differentiating decompensated heart failure from acute dyspnoea of other causes (McCulloch et al 2002). BNP has an exceptionally high negative predictive value (Cowie et al 1997). The concentrations correlate with the extent of ventricular dysfunction, the development of cardiac arrhythmia and the degree of haemodynamic compromise. High concentrations predict poor long term survival (Gottlieb et al 1989). BNP correlates with outcome most closely (Motwani et al 1993) and evidence that it specifically predicts sudden death has been used to suggest a role for BNP in selection of patients for internal cardiac defibrillator (Berger et al 2002). There is some evidence that serial BNP measurement may be useful as a guide for uptitration of heart failure treatment with more effective inhibition of the RAAS than is seen with more traditional prescribing (Murdoch et al 1999).

CNP predominates in the central nervous system, kidney and the vascular endothelium. Concentrations are very low in the plasma (Tawaragi et al 1990).

Recently a novel natriuretic peptide was identified from the venom of the green mamba snake *Dendroaspis angusticeps* and consequently named *Dendroaspis* Natriuretic Peptide (DNP) (Schweitz et al 1992). Immunoreactivity to this peptide has been

detected in human tissue and circulating levels have been demonstrated to correspond to the degree of heart failure (Schirger et al 1999). It is associated like the other natriuretic peptides with natriuresis and vasodilatation (Best et al 2002) but in animals at least appears to be resistant to degradation by NEP (Chen et al 2002)

The natriuretic peptides reduce effective circulating volume by increasing venous capacitance (Abraham et al 1998), natriuresis and diuresis (de Bold et al 1981). They increase renal blood flow and antagonise the RAAS (Gottlieb et al 1989, Wada et al 1994). ANP inhibits the production of catecholamines, renin, angiotensin II, aldosterone and ET-1 (Brands et al 1998, Atarashi et al 1984, Wada et al 1994) and suppresses ACE activity (Lang et al 1992). As a consequence there is reduced systemic vascular resistance, decreased intracardiac filling pressure and improvement in myocardial performance. There is a limit to cardiac remodelling (Wu et al 1997). The natriuretic peptides also cause vasodilatation by reducing sympathetic outflow and damping baroreceptor function (Yang et al 1992, Jin et al 1992, Currie et al 1983, Webb DJ et al 1998).

Interestingly, if the inhibitory effects of BNP and ANP on the RAAS are blocked in experimental heart failure, then it progresses more rapidly (Stevens et al 1995). This underlines the key role of the activated RAAS in the progression of heart failure and the favourable effects of natriuretic peptides in the condition.

The natriuretic peptides act through three receptors; A, B and C. The A receptor binds both ANP and BNP, but has a stronger affinity for ANP. ANP and BNP therefore have similar effects but ANP is the more important physiologically. This is reassuring when I consider the fact that natriuretic peptides are beneficial in heart failure but the effect of well established treatments is to lower the levels of BNP. They do not generally lower levels of ANP to the same extent.

CNP is the natural ligand for the B receptor and natriuretic peptide receptor C is involved in the clearance of the peptides (Koller and Goeddel 1992). Circulating natriuretic peptides are also inactivated by cleavage at the hands of NEP, present within renal tubular cells and vascular cells (Levin et al 1998). Renal dysfunction impairs clearance of the natriuretic peptides and results in higher plasma levels.

#### 1.2.4 BRADYKININ

Bradykinin is released from the inactive precursor, kininogen, by the enzyme kallikrein. Bradykinin is a strong vasodilator agent (Fox et al 1961). The activity of bradykinin is mediated via binding with one of two identified receptors B<sub>1</sub> and B<sub>2</sub>. Understanding of the role of these receptors in humans is limited. Animal in vitro studies have suggested that B<sub>1</sub> receptors are normally induced following vascular trauma, while B<sub>2</sub> receptors are expressed in endothelial cells (Drummond and Cocks 1995). B<sub>1</sub> receptors are said to have a role in chronic pathophysiological processes while B<sub>2</sub> receptors generally mediate most cardiovascular effects: hypotension, bronchoconstriction, plasma extravasation, regulation of smooth muscle tone, cell growth and acute inflammatory reactions (Schanstra et al 1998, Hall 1997, Marceau et al 1997, Kasel et al 1996, Schneck et al 1994).

Bradykinin is metabolised by kininase II, an identical molecule to that which activates angiotensin II from angiotensin I, ACE (Yang et al 1970) and NEP (Graf 1993). In fact when parenterally active ACE inhibitors first became available they were known as bradykinin potentiating peptides (Gavras et al 1974). It is not known to what extent endogenous bradykinin potentiation contributes to the therapeutic or adverse effects of ACE inhibitors but since the major regulator of bradykinin degradation in human endothelial cells is ACE it is certainly true that the hypotensive and other effects of ACE inhibitors in vivo may be due not only to inhibition of angiotensin II formation, but also to accumulation of bradykinin (Kasel et al 1996, Graf et al 1993).

It is possible that an increase in bradykinin activity is responsible for the relatively minor side effect of cough reported in a small number of patients and also the much more serious side effect of angioedema seen in a small but significant number of patients taking ACE inhibitors (Slater et al 1988, Kjekshus et al 1988, Israili and Hall 1992, Kostis et al 1994, 1996, Nussberger et al 1998, Agostoni et al 1999).

The effect on bradykinin activity of various neurohumoral modulators, and the association therefore with both therapeutic and potentially hazardous consequences, will be considered as part of this thesis. The implication that bradykinin may have a causative role in the potentially life threatening angioedema reported in a small number of patients taking omapatrilat is particularly interesting (Messerli and Nussberger 2000).

I look particularly at the effect on the bradykinin activity of the combined ACE/NEP inhibitor, omapatrilat and the combined ECE/NEP inhibitor, KC 12615.

#### 1.2.5 ADRENOMEDULLIN

Adrenomedullin (ADM) is an amino acid peptide with structural similarity to calcitonin gene-related peptide (CGRP) sufficient to allow cross reactivity at receptors (Bunton et al 2004). It was originally isolated from human pheochromocytoma. It is synthesized by a wide variety of tissues, including endothelial and vascular smooth muscle cells and acts as a circulating hormone and a local paracrine mediator. ADM production is up regulated by oxidative stress, pro inflammatory cytokines, angiotensin II and ET-1 (Chun et al 1997, Sugo et al 1995). Plasma concentrations of ADM are raised in heart failure in proportion to symptomatic and haemodynamic severity of the syndrome (Nishikimi 1995). They correlate with rising levels of natriuretic peptide but show a less brisk response to treatment – perhaps reflecting disease severity more closely. ADM may have a role as a prognostic indicator in heart failure (Pousset et al 2000, Richards et al 2001). ADM binds to and activates a specific ADM receptor and CGRP receptors (Beltowski and Jamroz 2004). It is a peptide with vasodilator, natriuretic and mitogenic actions (Eto et al 1999, Charles et al 1996) playing a significant role in endothelial regulation of blood pressure. Low dose infusion of ADM has been shown to reduce afterload, increase ejection fraction and increase cardiac index without an effect on heart rate in healthy volunteers (Del Bene et al 2000) and with diminished potency in patients with heart failure (Nakamura et al 1997). Its mechanism of action is thought to be a combination of intracellular effects on NO synthase, and possibly direct inhibition of both ET-1 production by endothelial cells (Hillier, Petrie et al 2001, Kohno et al 1996) and aldosterone production (Petrie et al 1999, Lainchbury et al 1999, Nagaya et al 2000).

The metabolism and clearance of ADM remains unclear. Since ADM shares structural similarities to the natriuretic peptides, NEP is a potential candidate. There is however conflicting data on the involvement of NEP in the degradation of ADM. Since an increase in natriuretic peptides levels has been directly associated with an increased plasma concentration of ADM and vice versa, interpretation of the data is complex (Bunton et al 2004). An effect of NEP inhibition on ADM degradation was demonstrated by Lisy et al in the dog (Lisy et al 1998) but inhibition of NEP was not



shown to augment ADM associated vasodilation, in the arteries of patients with heart failure (Petrie et al 2001). Further studies of the effects on ADM activity of NEP inhibition as well as vasopeptidase inhibition will be reported in this thesis.

#### 1.2.6 CALCITONIN GENE RELATED PEPTIDE

CGRP is another amino acid peptide with a potent vasodilating action (Brain et al 1985). CGRP and ADM bind to similar receptors and as such have similar effects (Beltowski and Jamroz 2004).

Increased levels of CGRP activity are found in chronic heart failure suggesting a role in the regulation of vascular tone and the pathophysiology of this condition (Gennari et al 1990). Finally, in human skin CGRP has been shown to cause persistent local reddening, thought to be secondary to dilatation of arterioles (Brain et al 1985). This raises the question of whether CGRP activity may be the mechanism by which some patients develop facial flushing as a side effect of vasopeptidase inhibition. Work looking at the effect of vasopeptidase inhibition on CGRP activity will be reported as part of this thesis.

#### 1.2.7 VASOACTIVE INTESTINAL PEPTIDE

Vasoactive intestinal peptide (VIP) is a 28-amino acid peptide. It is involved in autonomic regulation of the cardiovascular system, where it exerts positive inotropic and chronotropic effects. It is a potent vasodilator particularly associated with coronary vasodilatation (Henning and Sawmiller 2001) and reduced pulmonary vascular resistance. Heart failure has been associated with a reduction in circulating and myocardial VIP concentration or with alteration of affinity, density and physiological responsiveness of VIP receptors (Brodde et al 1992). ACE inhibitors have been shown to attenuate this depletion of VIP activity in heart failure (Duggan and Ye 1998) suggested as an additional mechanism for their positive inotropic effect. It is certainly possible that augmentation of VIP activity mediates some of the effects of vasopeptidase inhibition and for this reason it is included in my studies of their vasoactive effects.

### 1.2.8 SUBSTANCE P

Substance P is a member of the tachykinin family. Other members of this family include neurokinin A and neurokinin B. Its cardiovascular effects are largely mediated by the neurokinin receptor (Walsh and McWilliams 2006). The precise role of substance P in human physiology has been difficult to elucidate. It is suggested that it stimulates plasma extravasation in an endothelium dependent manner therefore playing a prominent role in tissue oedema and inflammation (Walsh and McWilliams 2006). Substance P also regulates vascular tone and therefore blood pressure and heart rate both peripherally and via the central nervous system (Dzurik et al 2007). It may relax vessels in some situations and constrict them in others, perhaps dependent on the condition of the endothelium. It is suggested that it has a complex perhaps otherwise compensated role in normal circumstances which becomes clinically apparent in disease.

As part of this thesis I consider the possibility that substance P mediates some of the benefits and perhaps the problematic side effects, for example angiooedema, associated with omapatrilat.

## **1.3 NEUROHUMORAL MODULATION**

### **– Vasoactive Enzyme inhibition**

It is now broadly accepted that the manipulation of neurohumoral pathways offers significant benefit to patients with cardiovascular disease, reducing both morbidity and mortality (Garg and Yusuf 1995, Flather et al 2000, Yusuf et al 2000, Fox 2003). During the development of now well established treatments, such as ACE inhibitors, we have learnt that inhibition of the activity of one vasoactive hormone often results in clinically important inhibition or potentiation of other pathways. Clearly there is value in antagonizing neurohumoral systems likely to have detrimental actions, and it is likely that the optimum strategy is to achieve this while simultaneously augmenting potentially desirable neurohumoral mediators. However enzyme inhibitors which act on multiple vasoactive mediators with complex interactions have unpredictable effects.

#### **1.3.1 THE RENIN ANGIOTENSIN ALDOSTERONE SYSTEM**

Blockade of the RAAS improves symptoms and survival in heart failure and ischaemic heart disease (CONSENSUS 1987, SOLVD 1991, Yusuf et al 2000, Fox 2003). Indeed greater inhibition has been shown to result in greater benefit (Packer et al 1999).

The current most popular approach to inhibition of this pathway is ACE inhibition. ACE inhibitors have been shown to improve mortality and morbidity in all classes of CHF, including asymptomatic left ventricular impairment post myocardial infarction (AIRE 1993).

ACE inhibitors reduce the generation of angiotensin II, a powerful vasoconstrictor, from angiotensin I by ACE. As a result of reduced angiotensin II stimulation of the adrenal cortex, aldosterone production is also reduced by ACE inhibition.

As ACE is also kininase II, ACE inhibitors have a second action, the reduction of the breakdown of bradykinin. ACE inhibition has been shown to increase bradykinin levels (Pellacini et al 1994). Whether this is a beneficial or detrimental effect remains unclear. While the hypotensive and other effects of ACE inhibitors in vivo may be due not only to inhibition of angiotensin II formation but also to accumulation of bradykinin, it is also suggested that an increase in bradykinin levels is responsible for a number of the side effects associated with ACE inhibitor therapy, side effects which not infrequently

result in the drug being discontinued. Cough is the most commonly reported cause of drug discontinuation and potentially life threatening angioedema is seen in a small but significant number of patients (Yusuf et al 2000, Fox et al 2003, Fuller and Choudry 1997). Both have been attributed to the rise in bradykinin activity in patients taking ACE inhibitors (Slater et al 1988, Kjekshus and Swedberg 1988, Israili and Hall 1992, Kostis et al 1994, 1996, Nussberger et al 1998, Agostoni et al 1999).

As a result of the inhibition of its break down to form angiotensin II, ACE inhibition also causes an accumulation of angiotensin I. Increased levels of angiotensin I may competitively overcome enzyme inhibition and lead to "renin-angiotensin-aldosterone escape" (Pitt et al 1995). Other enzymes, such as chymase, may continue to generate angiotensin II from angiotensin I (Urata et al 1990, McDonald et al 2001, Petrie et al 2001). It is known that the reduction in circulating aldosterone levels as a result of ACE inhibition is variable and often poorly sustained (Struthers 1996). This has been demonstrated in both the hypertensive and CHF populations (Biollaz et al 1982, Clelland et al 1984). In particular, over a third of heart failure patients are found to have elevated levels of aldosterone.

These and other considerations have led to the introduction and development of specific angiotensin II type 1 (AT<sub>1</sub>) receptor antagonists (Pitt et al 1995, 1997, 2000, Pfeffer et al 2003). The membrane bound AT<sub>1</sub> receptor mediates the detrimental effects of angiotensin II in heart failure. While these agents are undoubtedly effective in the treatment of cardiovascular disease, with improvement in CHF and post myocardial infarction morbidity and mortality comparable to that seen with ACE inhibitors (Pitt et al 1995, 1997, 2000, Pfeffer et al 2003), angiotensin II receptor antagonists have been shown to result in accumulation of angiotensin II and the consequences of this, through the actions of angiotensin II at other receptors, are unknown (Gottlieb et al 1993, Cao et al 1999).

Since it has been demonstrated that ACE inhibitors and angiotensin II receptor antagonists, while clearly offering considerable benefit in chronic heart failure offer only partial inhibition of the deleterious RAAS, it is not unexpected that large studies have been performed considering the addition of RAAS antagonism at its origin and its end point.

The aldosterone antagonist, spironolactone, has been studied widely in the chronic heart failure population and has been shown to reduce mortality and morbidity in patients with NYHA stage III and IV symptoms (Pitt et al 1999).

Renin cleaves circulating angiotensinogen to angiotensin I which is then transformed into angiotensin II. This is the rate limiting step in the formation of angiotensin II (Fisher and Hollenberg 2001, Nussberger et al 2002). A functional membrane bound renin receptor has also been characterised (Nguyen et al 2002) and it has been suggested that receptor bound renin is able to activate angiotensinogen with a higher efficiency than renin in solution.

Renin has very high substrate specificity; it's only known substrate is angiotensinogen. In theory therefore renin inhibitors should specifically and efficiently block the formation of angiotensin II.

Renin inhibitors should also have a therapeutic profile distinct from ACE inhibitors and angiotensin receptor antagonists. ACE inhibitors reduce the conversion of angiotensin I to angiotensin II and angiotensin receptor antagonists prevent binding of angiotensin II to the AT<sub>1</sub> receptor. Both these inhibitors, however, also interrupt the normal feedback suppression of renin secretion from the kidneys, causing reactive rises in circulating renin, angiotensin I (in the case of ACE inhibitors) and angiotensin II (in the case of angiotensin receptor antagonists) levels (Hollenberg et al 1998). In the case of ACE inhibitors, the rise in circulating angiotensin I may lead to an increase in the formation of angiotensin II via pathways independent of ACE (e.g. chymase) or even overcome the blockade of ACE. We know that beta blockers which reduce the release of renin are not associated with such escape processes (Buhler et al 1972). With angiotensin receptor antagonists, the reactive increase in angiotensin II may stimulate other angiotensin receptor subtypes, with unknown consequences. ACE inhibitors are of course also non substrate specific, inhibiting the breakdown of bradykinin and possibly other substances.

As long ago as 1957, the potential benefits of specific inhibition of the renin system by diminishing renin activity and not other pathways, has been highlighted (Skeggs et al 1957). Until recently, the introduction of renin inhibitors into clinical practice was limited by low oral bio-availability, poor efficacy, short duration of action and high cost

of chemical synthesis (Fisher and Hollenberg 2001, Nussberger et al 2002). These problems have in part been overcome with the development of the new, orally active, specific renin inhibitor, aliskiren (Nussberger et al 2002). The first work looking at the haemodynamic and neurohumoral effects of renin inhibition in patients with chronic heart failure is reported as part of this thesis.

### 1.3.2 NEUTRAL ENDOPEPTIDASE INHIBITION

The second major class of agents, to reach an advanced stage of clinical development, with the ability to regulate the production and degradation of vasoactive factors, was the NEP inhibitors.

The success of ACE inhibitors has encouraged the development of alternative approaches to the blockade of the RAAS. It is acknowledged that ACE inhibition is poor at renin-angiotensin-aldosterone suppression in the long term (Rousseau et al 1994, Kirlin et al 1995). NEP inhibition may offer us such an approach as well as augmenting the potentially beneficial natriuretic peptides.

NEP is a plasma membrane bound metalloprotease. It catalyzes the degradation of a number of endogenous vasodilator peptides, including ANP, BNP, CNP (Stephenson and Kenny 1987, Lang et al 1992, Kenny et al 1993), substance P (Skidgel et al 1984) bradykinin (Graf et al 1993) and possibly ADM. There are conflicting data regarding ADM degradation, with augmentation of ADM activity reported in dogs (Lisy et al 1998) but no effect of NEP inhibition on ADM activity seen in humans (Petrie et al 2001).

Inhibition of the degradation of the natriuretic peptides, has been shown in vitro and in animal models to inhibit the RAAS in several ways, reducing the synthesis of renin and aldosterone and suppressing ACE activity (Brands and Freedman 1988, Atarashi et al 1984, Lang et al 1992). These results support the suggestion that NEP inhibition is an attractive therapeutic option.

Candoxatrilat is an orally active, selective NEP inhibitor. Sinorphan, a pro-drug of thiorphan, is also a selective NEP inhibitor. Both agents have been administered to humans with encouraging effect on circulating ANP levels and natriuresis but disappointing effect on blood pressure (Richards et al 1992). Systemic NEP inhibitor

administration did not cause reduction in the blood pressure of normotensive individuals (O'Connell et al 1992) and indeed there are reports of the blood pressure increasing in response to candoxatrilat. The lowering of blood pressure in hypertensive patients although reported was by no means universal (Richards et al 1993, Favrat et al 1995).

These results were disappointing and prompted further work into the in vivo activity of NEP. While there is evidence that the vasodilator peptides are augmented in the presence of NEP inhibition, it has now been suggested that the predominant physiological substrates for vascular NEP are vasoconstrictor peptides and particularly ET-1 (Ferro et al 1998), explaining the contradictory haemodynamic results obtained after systemic dosing with NEP inhibitors.

Infusion of thiorphan into the human forearm, leading to local NEP inhibition, causes vasoconstriction at least in part reversed by a co-administration of an ET receptor antagonist (Plumpton et al 1995, Haynes and Webb 1994, Love et al 1996, Ferro et al 1998). Candoxatril also increases plasma ET-1 concentrations (Ando et al 1995, McDowell et al 1997). Both these observations suggest that NEP may contribute to degradation of ET-1 (Russell et al 1996). There is some evidence, in guinea pig and rat lung, that NEP can also convert big ET-1 to the active ET -1 (Murphy et al 1994, Held et al 1997, Lebel et al 1996). Local vascular accumulation of ET-1 may counteract the blood pressure lowering effect of an increase in plasma natriuretic peptide concentrations after NEP inhibition, at least in healthy subjects and hypertensives (Ando et al 1995, Richards and Crozier et al 1992, Ferro et al 1998, McDowell et al 1997). NEP may also degrade angiotensin II and this too may counteract the hypotensive effect of natriuretic peptides (Richards and Wittert et al 1992, Richards et al 1993). NEP inhibition alone seemed to be more promising in CHF, compared to hypertension, possibly because of the much greater effect on circulating natriuretic peptide concentrations in this condition (Newby et al 1998, Northridge et al 1999). Even in CHF, however, a vasoconstrictor response to systemic NEP inhibition has been observed (Kentsch et al 1996).

The physiological actions of NEP are presumed to be the result of a balance between the degradation of the vasoconstrictor and the vasodilator hormones (see figure 1.3.1). It is understood therefore that the effects of NEP inhibition are complex.

One possible solution to this is combined or dual enzyme inhibition. It seems reasonable to suggest that inhibition of NEP while at the same time inhibiting the vasoconstrictor pathways independently, for example the RAAS with ACE inhibition, or the ET system with ECE inhibition, should result in a more effective therapeutic strategy. Favrat and associates have shown that a combination of an ACE inhibitor, captopril, and a NEP inhibitor, sinorphan, results in a greater antihypertensive effect than either agent alone (Favrat et al 1995). Much of the work described in this thesis looks at two recently developed molecules which combine ACE and NEP inhibition, and ECE and NEP inhibition, omapatrilat and KC 12615 respectively.

### 1.3.3 VASOPEPTIDASE INHIBITION

The RAAS and natriuretic peptides have been the targets for new interventions over the past two decades. More specifically targeted are the enzymes responsible for regulation of the synthesis and degradation of the major peptides involved (Figure 1.3.1). A number of molecules which inhibit both ACE and NEP, known as vasopeptidase inhibitors, have been synthesized and are at various stages of clinical development. While there has been some cause for concern regarding the adverse effects of these molecules, it is acknowledged that there are likely benefits in dual enzyme inhibition, perhaps in a selected population (Coats 2000, 2002, Campbell 2003, Richards et al 2003).

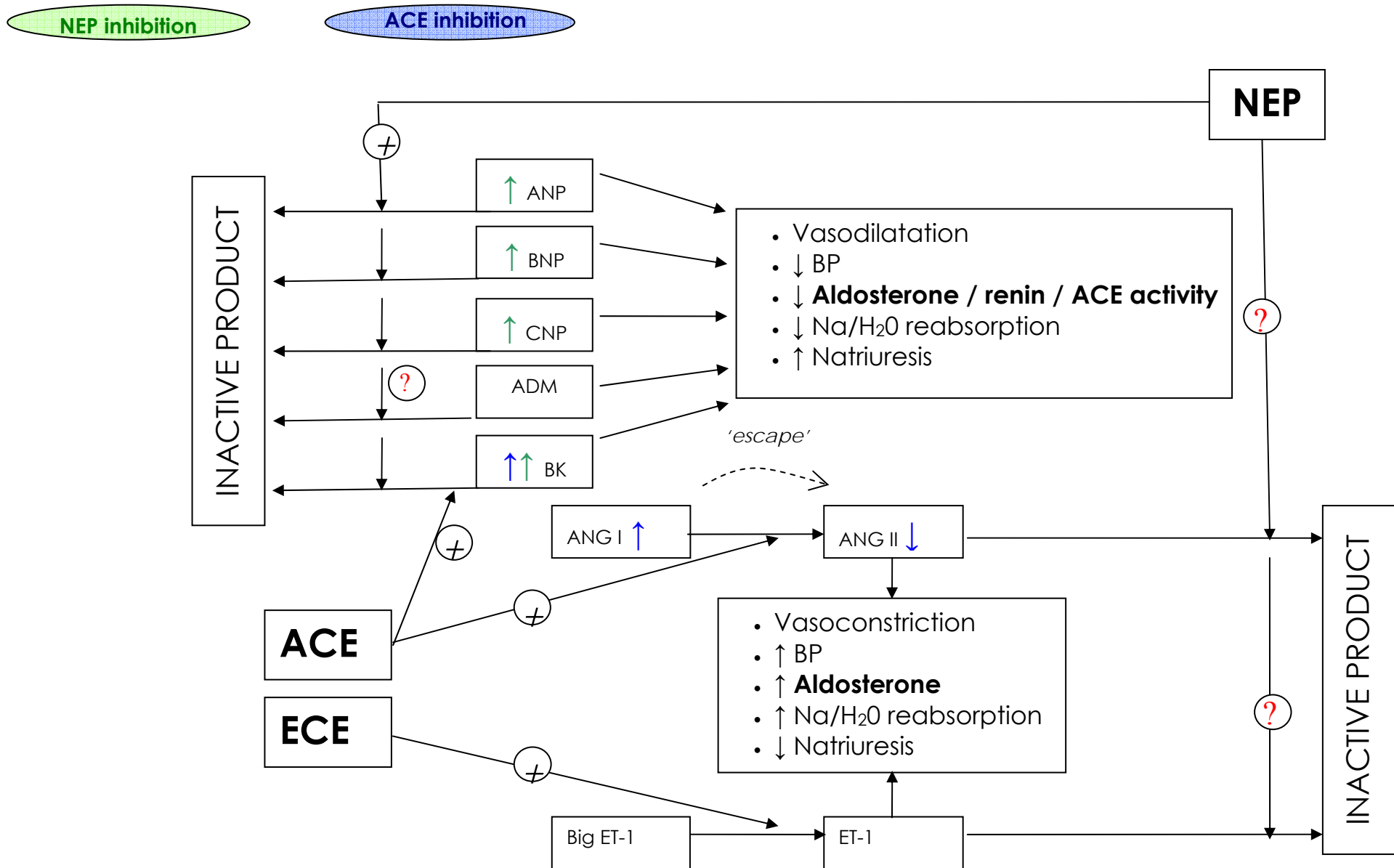
Omapatrilat (BMS 186716) (figure 1.3.2) is the most clinically advanced agent in this class having been widely studied in both heart failure and hypertension in randomised controlled clinical trials. It was the first orally active molecule available and is a potent and specific inhibitor of both ACE and NEP activity,  $K_i$  of 6 and 9nmol/L respectively (Trippodo et al 1998). Other molecules have also now been given to humans (table 1.3) with encouraging effect on blood pressure when compared to ACE inhibitor or placebo in a hypertensive population.

I report on the NEP and ACE inhibitory properties of one of the more recently developed molecules, GW660511X ('511) as part of this thesis. The pharmacodynamic profile of the ACE and NEP inhibitor activity of '511 has not been fully described in humans. Initial studies have characterized the ACE inhibition by assessing plasma ACE



**Figure 1.3.1**

The pathways affected by vasoactive enzyme inhibition. ↑ = augmented, ↓ = inhibited



**TABLE 1.3 PUBLISHED STUDIES OF HAEMODYNAMIC EFFECTS OF VASOPEPTIDASE INHIBITORS**

Vasopeptidase inhibitor (reference)	Number of patients	Population Mean age (yrs)	Placebo / active control	Doses used (number receiving)	Means of administration Duration of therapy (observation)	Reduction in blood pressure from baseline with highest dose (mmHg)  Systolic (S) Diastolic (D) Mean arterial (M)
<b>Sampatrilat</b> (Norton et al 1999)		Hypertensive 50	Lisinopril	Lisinopril 10-20mg (30) Sampatrilat 50-100mg (28)	Oral 56 days	S: <b>8</b> D: <b>5</b>
<b>MDL 100240</b> (Rousso et al 1999)	12	Healthy volunteers 24	Placebo	Placebo MDL 100240 6.25mg MDL 100240 25mg	Intravenous Single dose (24hrs)	S: <b>8</b> D: <b>3</b>
<b>Z13752A</b> (Bani et al 2000)	16	Healthy volunteers 25	Placebo	Placebo Z13752A 10-800mg	Oral Single dose (24hrs)	<b>No significant change</b>
<b>Fasidotril</b> (Laurent et al 2000)	57	Hypertensive 50	Placebo	Placebo Fasidotril 100mg bd	Oral 42 days	S: <b>18</b> D: <b>10</b>
<b>Mixanprilat / S21402</b> (Chodjania et al 2002)	10	Healthy volunteers	Captopril	Captopril 50mg Mixanprilat 250mg	Oral Single dose	<b>No significant change</b>
<b>GW660511X</b> (Johnson et al 2006)	123	Hypertensive 51	Placebo	Placebo (62) GW660511X 200mg (61)	Oral 14 days	S: <b>8</b> D: <b>5</b>
<b>Ilepatril / AVE 7688</b> (Azizi et al 2006)	24	Healthy volunteers	Ramipril Irbesartan	Placebo Ramipril 10mg Irbesartan Irbesartan and ramipril Ilepatril 5mg Ilepatril 25mg	Oral Single dose (48hrs)	M: <b>12</b>

activity and have partially characterized markers of NEP inhibition. I compare it with an active control in a healthy population.

Molecules which inhibit NEP, ACE and ECE are also in development but as yet not given to humans (Battistini et al 2005).

### OMAPATRILAT

Administration of omapatrilat results in dose dependent increase in plasma ANP, plasma renin activity, plasma bradykinin levels and the urinary excretion of ANP and cGMP, characteristic of NEP inhibition (Hammett et al 1999, Vesterqvist et al 1999, McLean et al 2000, Campese et al 2001). Omapatrilat administration also results in a reduction in serum ACE activity despite an increase in plasma renin activity (Vesterqvist et al 1997, 1999, Campese et al 2001).

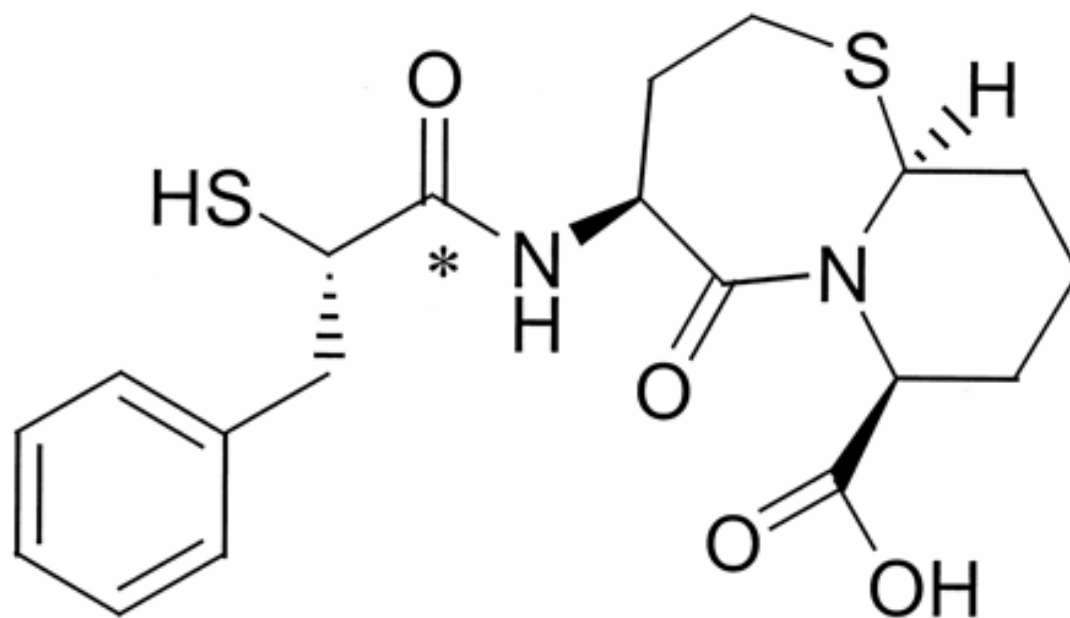
A reduction in circulating levels of angiotensin II and therefore aldosterone, and augmentation of the natriuretic peptides, would be expected to lead to a reduction in vascular tone and therefore a reduction in blood pressure. The reported effects on blood pressure, of omapatrilat compared with placebo, are encouraging, with a number of groups reporting significant reduction in systolic and diastolic pressure (Ruddy et al 1999, Zusman et al 1999, Aronoff et al 2000, Larochelle et al 2000, Mitchell et al 2002). The challenge for the development of vasopeptidase inhibitors, as useful therapy in hypertension or heart failure, has been the need for demonstration of a beneficial effect over and above ACE inhibition. More significant, therefore, are those studies which demonstrate that omapatrilat results in greater blood pressure reduction than the ACE inhibitors, lisinopril and enalapril in patients with hypertension. (Campese et al 2001, Ferrario et al 2002, Mitchell et al 2002, Kostis et al 2004).

One of these groups also reported a previously unknown property of omapatrilat. Vesterqvist et al noted that administration of omapatrilat resulted in an increase in ADM levels (2000). This could be explained by the theory that NEP is involved in the degradation of ADM, supporting the results of animal work by Lisy et al (1998). As previously described there are contradictory reports on this role (Petrie et al 2001).

When compared with placebo, omapatrilat has also been associated with improved ejection fraction, and reduced systolic and diastolic blood pressure in heart failure

**Figure 1.3.2**

The chemical structure of omapatrilat



patients, as well as increased urinary volumes and urinary excretion of sodium and ANP, demonstrating inhibition of NEP. Again however, the effects of omapatrilat, need to be compared with the effects of ACE inhibition alone in patients with heart failure.

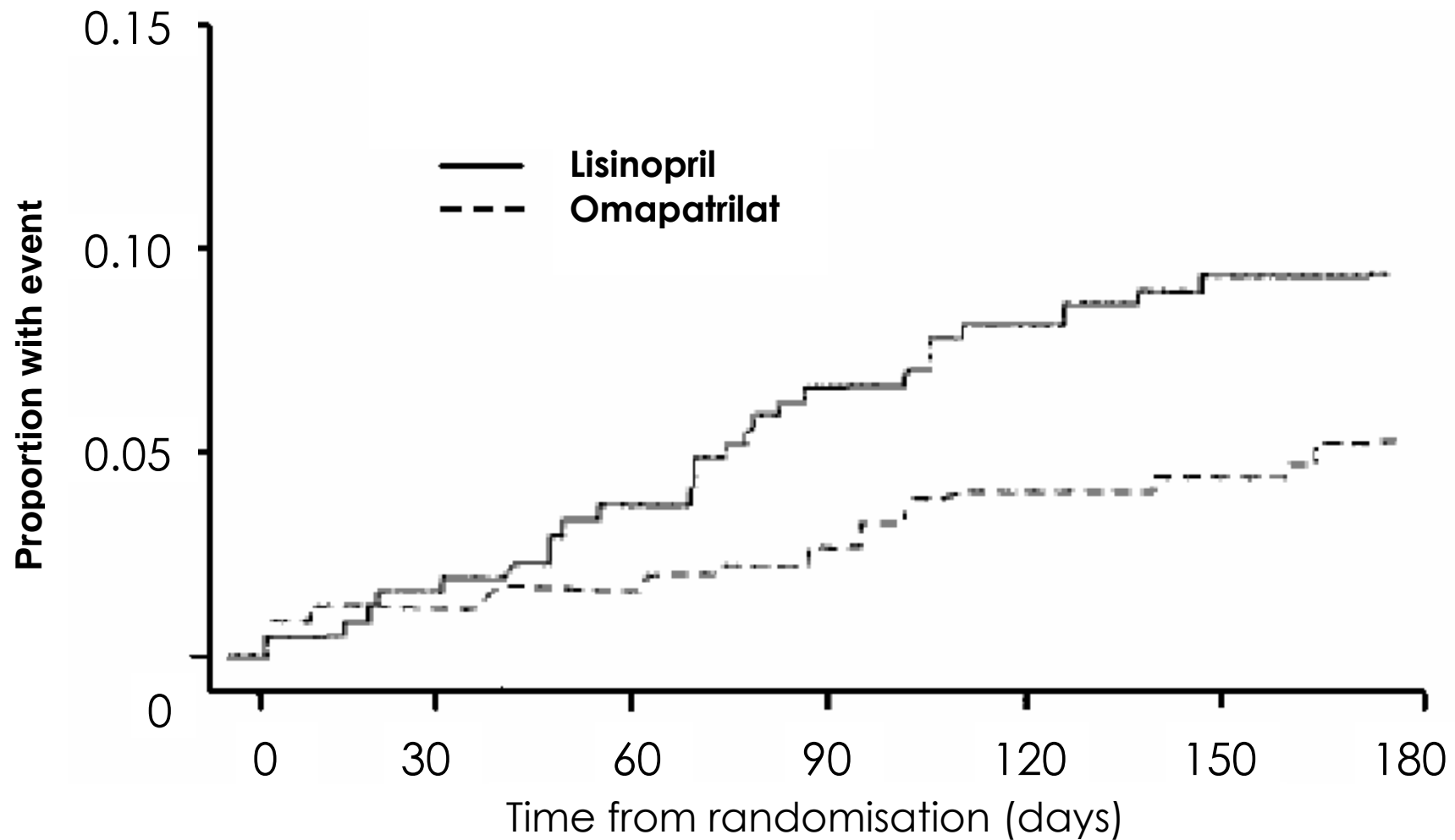
IMPRESS was a randomised study comparing the effects of omapatrilat and lisinopril in patients with heart failure over a period of 24 weeks (Rouleau et al 2000). 573 patients were randomised. The primary end-point was change in exercise tolerance but morbidity and mortality were also reported. All patients enrolled were required to be on an ACE inhibitor, a selected population therefore, but ACE inhibitor therapy was stopped prior to randomization.

Importantly, in the primary end point, exercise tolerance, there was no difference between the two groups. More positively, the reported difference in mortality and hospital admission rates between the two patient groups did suggest benefit of omapatrilat over lisinopril, the Kaplan-Meier curve for the composite of death or admission for worsening heart failure, separating early but not reaching significance by the end of the 24 week period ( $p=0.052$ , figure 1.3.3) At the end of week 24 omapatrilat treatment had also led to more improvement and less worsening of NYHA class, in patients in NYHA class III or IV (figure 1.3.4), although not significant when patients in NYHA II were included. Overall these results were encouraging and led Bristol-Myers-Squibb, the manufacturer and patent holder, to file a new drug application with the FDA.

Unfortunately subsequent studies have been less well received. The new drug application for omapatrilat was voluntarily withdrawn in April 2000 because of reports of a significant number of cases of angioedema in patients taking omapatrilat. Initially it was hoped that the incidence of this potentially life threatening adverse event would compare well to that seen and accepted in patients taking ACE inhibitors. In fact the OVERTURE study published in 2000, confirms that the incidence of angioedema is significantly higher in patients taking omapatrilat, when compared to those taking ACE inhibitors. Angioedema was reported in 24 (0.8%) omapatrilat-treated and 14 (0.5%) enalapril-treated patients. Having been exposed to the drug for a relatively short period of time this apparently low incidence is very significant. The incidence of angioedema overall was however less than that previously reported in hypertension studies and it was suggested by the investigators that heart failure patients may be less vulnerable to

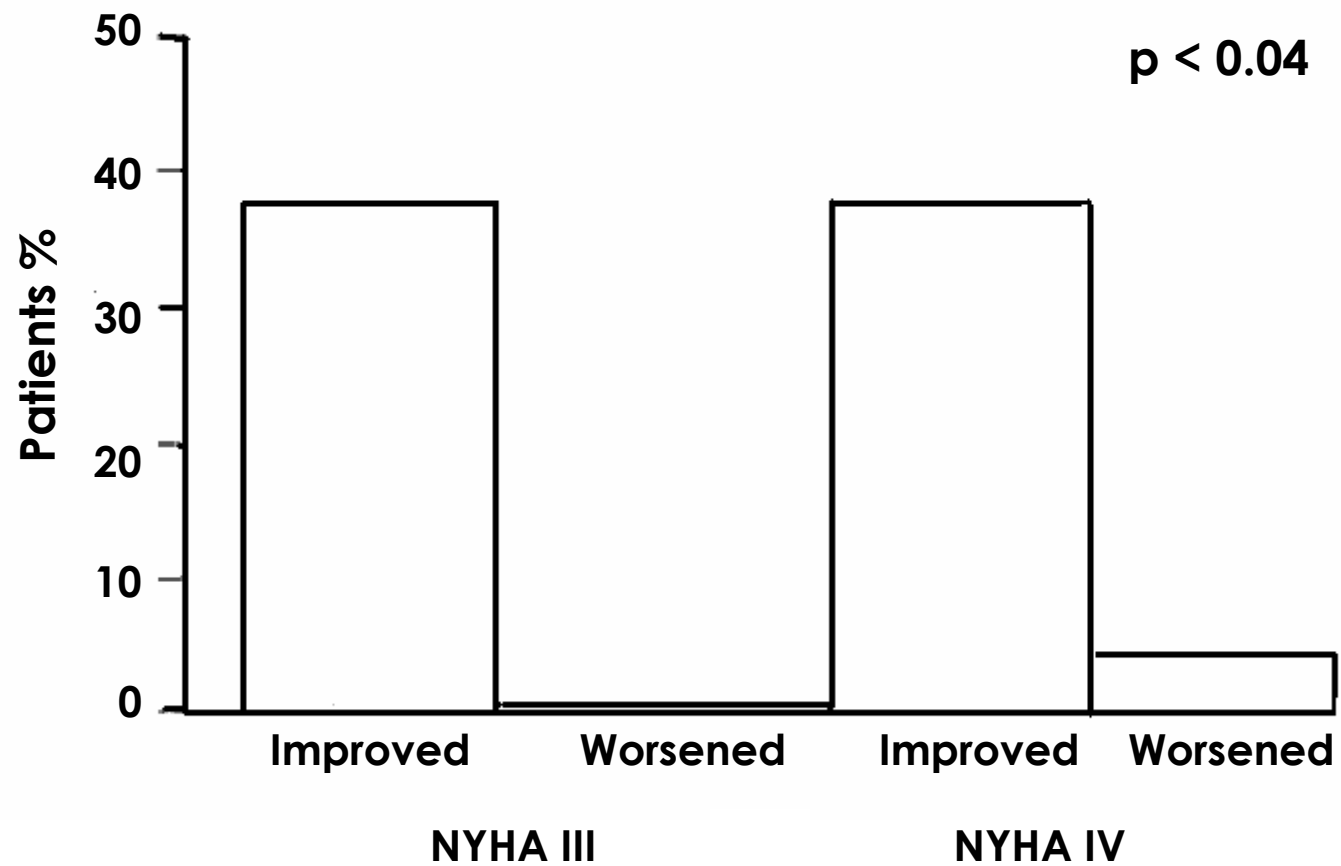
**Figure 1.3.3**

The IMPRESS study. Kaplan-Meier curve for the composite of death or admission for worsening heart failure, separating early but not reaching significance by the end of the 24 week period (p 0.052)



**Figure 1.3.4**

The IMPRESS study. The effect of omapatrilat on NYHA class in patients in NYHA class III and IV



the effects of bradykinin augmentation (Packer et al 2002). CHF also carries a more serious prognosis than hypertension and risk/benefit ratios of therapeutic interventions must be assessed separately for the two indications (Willenheimer and Swedberg 2000).

The mechanism by which patients taking omapatrilat develop angioedema is not fully understood. It is three times more common when a starting dose of 20mg is used than it was with lower doses, suggesting a pharmacodynamic rather than an allergic effect. Bradykinin is probably the mediator of the angioedema associated with ACE inhibitors (Nussberger et al 1998, Agostoni et al 1999, Israili and Hall 1992) and since we know NEP also metabolises bradykinin it is perhaps not unexpected that a combined ACE/NEP inhibitor would be associated with a higher incidence of angioedema.

Aside from the angioedema story, the results of this study were not as impressive as had been hoped for following the IMPRESS trial. OVERTURE was designed to compare the efficacy of omapatrilat to that of an ACE inhibitor, enalapril, in patients with heart failure, encouraged by the positive findings of the IMPRESS trial and studying patients over a longer period. In fact, it reported no significant superiority of omapatrilat in the primary end point of all cause mortality or hospitalization for heart failure. On a more positive note, with regard to overall adverse event profile, both drugs were well tolerated in moderate to severe heart failure and interestingly, renal failure was less common in the omapatrilat group despite more reports of significant hypotension. This is consistent with previous reports that NEP inhibition has a protective effect on renal function particularly in the context of ACE inhibitor use (Chen et al 1999, Troughton et al 2000). The effects of NEP inhibition on renal function are thought to be due to an increase in glomerular filtration rate and renal blood flow, they are thought to be less significant in severe heart failure in which there is thought to be a degree of resistance to ANP mediated natriuresis (Chen et al 1999).

The most recently published study OCTAVE (Kostis et al 2004) is a study in hypertension. A multicentre, randomised, double blind study over 24 weeks, it compared omapatrilat with enalapril as first line replacement for existing inadequate treatment or in addition to existing therapy in more than 25,000 patients with hypertension. It was primarily designed to see if starting with smaller doses of omapatrilat would result in a lower incidence of angioedema in this population.



Omapatrilat reduced systolic blood pressure by 3.6mmHg more than enalapril at week 8 and was associated with less use of adjunctive therapy by week 24 (19% vs 27%;  $p<0.001$  for both comparisons). In all three groups, patients treated with omapatrilat were more likely to reach target blood pressure. Angioedema however was still more frequent with omapatrilat (2.17% vs 0.68%), it occurred more quickly and was more likely to be severe. The risk of requiring hospitalisation 9.5 times higher in the omapatrilat group with two patients suffering airway compromise that was successfully treated.

Overall in a setting resembling clinical practice omapatrilat provided broadly superior antihypertensive efficacy when compared to ACE inhibitor. However potentially life threatening side effects unlikely to be diagnosed and treated as effectively in clinical practice as they are in highly controlled clinical studies remain a real concern.

Overall, it would seem that omapatrilat is an effective antihypertensive agent. Its superiority to the ACE inhibitors, lisinopril and enalapril in this population perhaps not fully explained by what we know already with regard to NEP inhibition. It is not however superior to enalapril in heart failure patients, contrary to what we expected following neurohumoral and pilot studies. Patients with hypertension and heart failure may be an exception to this, if subgroup analysis is to be considered (Kostis et al 2004). Omapatrilat apparently does reduce morbidity and mortality more than enalapril in this population to an extent consistent with its antihypertensive efficacy. Finally and most significantly however, omapatrilat carries a significantly higher risk of the serious adverse event, angioedema in both populations, the mechanism of which is not well understood.

With the OCTAVE results available to them in 2002 the FDA, again on balancing risk versus benefit, refused Bristol-Myers-Squibb application for a new drug licence. It would seem that this is the end of the omapatrilat story. Certainly OPERA (Omapatrilat in Persons with Enhanced Risk of Atherosclerotic Events, Kostis et al 2002)) which proposed to compare omapatrilat with placebo in 12,600 patients with borderline hypertension over five years has subsequently been dropped apparently due to poor recruitment.

Neurohumoral modulation undoubtedly has much to offer in the treatment of cardiovascular disease. More work looking at molecules that inhibit both ACE and NEP is clearly merited, if only to increase our understanding of the complex actions of these enzymes. In a selected population, particularly patients with renal dysfunction early in their heart failure course, or patients with resistant or complicated hypertension, there may be a role for omapatrilat. Unfortunately its role in the unselected hypertension population and perhaps even in unselected heart failure patients is seriously threatened

by a significant risk of life threatening side effect. Further studies of the mechanism of the side effects is required and the work described in this thesis contributes to the debate.

#### 1.3.4 THE ENDOTHELIN SYSTEM

Endothelin-1, the most important ET isopeptide in the human cardiovascular system, is a powerful vasoconstrictor and has renal, mitogenic and other actions to suggest that it might play an adverse role in CHF, hypertension and atherosclerosis (Grantham et al 1998, Kedzierski et al 2001). It is a powerful vasoconstrictor; demonstrated to have ten times the vasoconstrictor potency of angiotensin II (Yanagisawa et al 1988). There are at least two distinct endothelin receptors; ET A and ET B. ET A receptors are found on the vascular smooth muscle cells and mediate vasoconstriction (Seo et al 1994). ET B receptors are found on both vascular smooth muscle cells mediating vasoconstriction and endothelial cells. They mediate endothelial cell release of NO and prostacyclin to mediate vasodilatation (Tsukahara et al 1994). In addition binding to ET B receptors represents the primary clearance mechanism for ET-1, particularly in the pulmonary and renal vascular beds (Dupuis et al 1996, Bohm et al 2003). The balance between ET A and ET B receptor activation undoubtedly plays a key role in the regulation of vasomotor tone. Like ACE inhibitors and angiotensin receptor antagonists, ET receptor antagonists might also improve prognosis in chronic heart failure. Indeed in experimental heart failure, they have been shown to improve survival (Sakai et al 1996). In patients with chronic heart failure both non selective and selective ET receptor antagonists reduce pulmonary and systemic vascular resistance and increase cardiac output (Kioski et al 1995, Sutsch et al 1998).

A number of endothelin receptor antagonists have been studied in patients with heart failure, including BQ 123, BQ 788, BMS193884, TBC 11251 (sitaxsentan), LU 135252 (darusentan), Ro-61-0612 (tezosentan) and bosentan (Ro 47-0203). (Love et al 1996, 2000, Cowburn et al 1998, Smith et al 2000, Torre-amione et al 2001, Givertz et al 2000, Spieker et al 2000, Kiowski et al 1995).

Bosentan is a non-selective ETA/B antagonist, the most widely studied of this class. The REACH-1 (Research on Endothelin Antagonism in Chronic Heart failure trial, Packer et al 1998, 1999) studied the effect of prolonged treatment with Bosentan. This multi-centre, double-blind, placebo-controlled study assessed the effect of bosentan therapy for 6 months in patients with advanced heart failure. 370 patients with severely symptomatic chronic heart failure despite treatment with diuretics, digoxin and an ACE inhibitor were randomised to receive either bosentan (target dose 500 mg twice daily) or placebo. The primary endpoint of the study was a clinical composite which assessed symptoms and major events. The trial was stopped early because of an excess of asymptomatic, reversible elevations in hepatic transaminases and reductions in haematocrit in the bosentan group. In the entire study population, there was no significant difference between bosentan and placebo. However, only 173 patients (47%) had been followed for 6 months at the time of trial discontinuation. In the subgroup of patients followed for the intended 6 months duration of the study, bosentan significantly increased the likelihood of clinical improvement and decreased the likelihood of symptomatic deterioration. Furthermore, bosentan reduced the total number of hospitalisations for any reason by 41%. The benefits of bosentan seemed to increase with longer duration of therapy. The REACH-1 trial was not powered to detect any effect on mortality.

More recently the ENABLE (Endothelin Antagonist Bosentan for Lowering Cardiac Events in Heart Failure) study (Coletta et al 2002) evaluated the effects of a lower dose of bosentan, again in patients with severe heart failure. A total of 1,613 patients were randomized to receive either bosentan (125 mg twice a day) or placebo. The preliminary results were presented in 2002. The primary endpoint of all-cause mortality or hospitalization for heart failure was reached in 321/808 patients on placebo and 312/805 receiving bosentan. Treatment with bosentan appeared to confer an early risk of worsening heart failure necessitating hospitalization, as a consequence of fluid

retention. It has been suggested that further studies using even lower doses of bosentan or more aggressive concomitant diuretic therapy may avoid this adverse effect.

The results from the REACH and ENABLE studies have undoubtedly thrown considerable doubt on the potential benefits of non-specific endothelin receptor blocker in chronic heart failure. Further studies have been carried out using a similar molecule but in the acute setting.

Tezosentan is an intravenously administered competitive antagonist of both ET A and ET B receptors. As expected by inhibiting the effects of endothelin, it is a powerful vasodilator. The haemodynamic and clinical effects of tezosentan were studied in a series of large, double blind phase III studies, collectively known as the RITZ program. Patients with acute heart failure were treated with doses of 50-100mg/hr of tezosentan (Teerlink et al 2001, Torre – Amione et al 2003, O'Connor et al 2002, Kaluski et al 2003). The results of the RITZ program while demonstrating haemodynamic benefit did not report clinical benefit at these doses. Further analyses of the results suggested that the doses used were at the top end of the dose response curve, causing an excess of side effects and, perhaps as a result of massive vasodilatation, limited efficacy.

In a more recent phase II study, lower doses of tezosentan were used (0.2-25mg/hr), again in patients with acute heart failure who had failed to respond to intravenous diuretic therapy (Cotter et al 2004). Interestingly the overall haemodynamic response to these lower doses was found to be similar in magnitude to that seen at high dose but with a later onset and more prolonged duration. Only a trend toward improvement in patient symptoms was observed but a significant decrease in BNP levels, previously associated with improved outcome over the long term (Cheng et al 2004), was noted.

It is suggested that, while higher doses of endothelin antagonist cause haemodynamic effects associated with excessive vasodilatation, decreased urine output and increased plasma endothelin levels in acute heart failure, lower doses may result in similar reduction in pulmonary wedge pressure but with longer term benefit and without the same adverse effects. This most recent study however is but a preliminary study, having recruited only a small number of patients (n=130) and therefore its interpretation has to be limited.

Further large studies are eagerly awaited, due to report late in 2007, looking particularly at a 1mg/hr dose and its effect on clinical outcome. The VERITAS program consists of 2 identical, double-blind, randomized, placebo-controlled, concurrently conducted trials (VERITAS-1 and VERITAS-2), performed in 150 centers in Europe, Israel, Australia, and North America (Teerlink et al 2005). The program is designed to enroll at least 1760 patients hospitalized with dyspnoea at rest because of acute heart failure, requiring intravenous therapy. In addition to conventional therapy, patients are randomized to receive tezosentan (5 mg/h for 30 minutes, then 1 mg/h for 24-72 hours) or matching placebo. The 2 prespecified primary end points are the incidence of death or worsening heart failure at 7 days in the combined studies and the change from baseline in dyspnea over the first 24 hours of treatment, measured using a visual analog scale in VERITAS-1 and VERITAS-2, individually. Since no currently available agents have been shown in a prospective, randomized, clinical trial to improve outcomes in patients with acute heart failure, the VERITAS program will provide valuable insights into the effect of tezosentan on clinical outcomes as well as hemodynamics and clinical symptoms.

Since selective ET B receptor blockade worsens haemodynamic variables in chronic heart failure (Wada et al 1997), selective ET A receptor antagonists might be thought preferable to mixed ones. EARTH, Endothelin Receptor Antagonist Trial in Heart Failure, is a multicentre randomized, double blind, placebo controlled, parallel, dose ranging study looked at the long term effects of different doses of the orally active ET A antagonist, darusentan on left ventricular remodeling, neurohormones and symptoms in patients with advanced chronic heart failure (Anand et al 2004). They were also unable to demonstrate benefit. Six months of treatment failed to improve left ventricular remodeling and offered no clinical benefit at any dose. This was despite a reduction in blood pressure and an increase in circulating endothelin levels suggesting effective receptor blockade.

The EARTH investigators suggest that the background treatment of the heart failure population is particularly relevant. That ACE inhibition or angiotensin receptor antagonists, beta blockade and spironolactone, taken by 95%, 73% and 37% of their patients respectively might already have caused maximal remodeling effects.

In summary neither ET A selective or non selective receptor antagonists have shown therapeutic benefit in chronic (bosentan in ENABLE, darusentan in EARTH) or acute

(tezosentan in RITZ) heart failure. While the results of VERITAS remain outstanding the emphasis in this class has understandably moved away from the CHF population concentrating instead on other pathology such as pulmonary hypertension and renal disease (Channick et al 2001, Neuhofer et al 2006, Benza et al 2007). Perhaps a subgroup of heart failure patients with these common comorbidities will ultimately find benefit.

Endothelin converting enzyme (ECE) converts big ET to ET. SLV 306 is a dual metalloprotease inhibitor, inhibiting both NEP and ECE. An alternative inhibitor of the endothelin system, it is in phase II of development (figure 1.3.5).

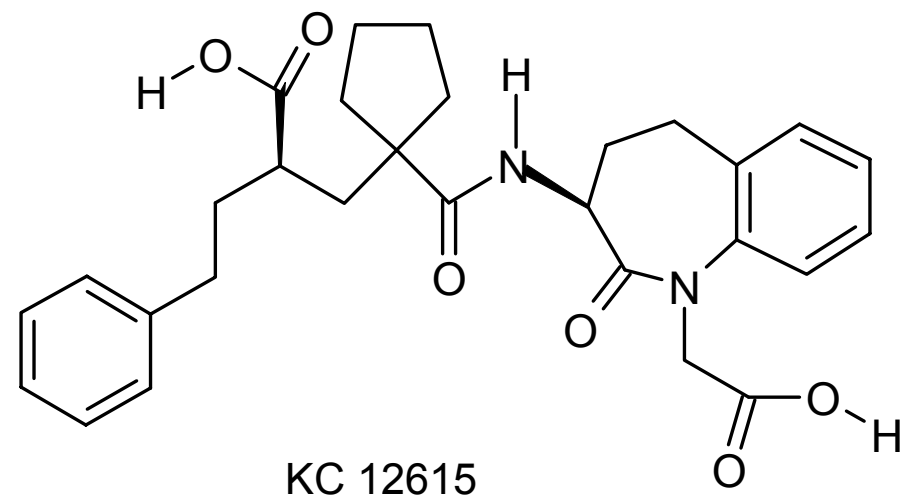
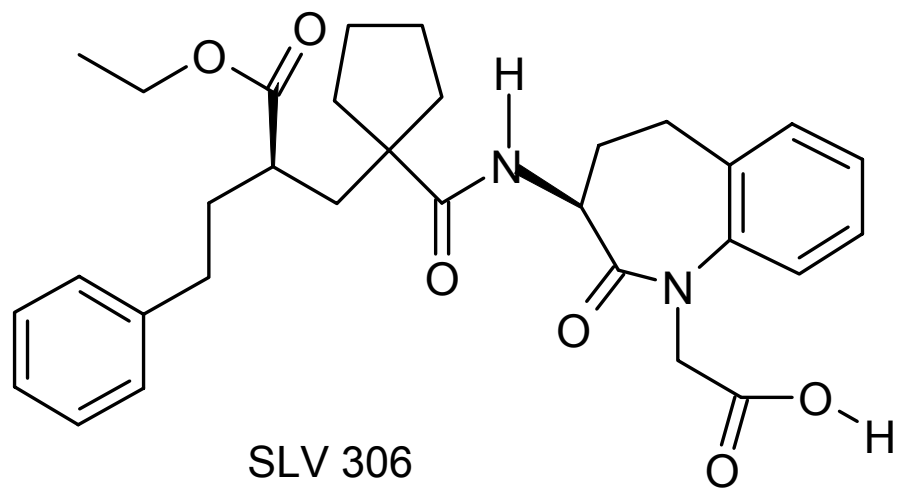
Combined NEP and ECE inhibition, leading to augmented natriuretic peptide and bradykinin concentrations, coupled with reduced ET-1 synthesis is an attractive therapeutic strategy in a range of cardiovascular diseases. The natriuretic peptides ANP, BNP and CNP are anti-mitogenic, natriuretic and vasodilatory peptides. Augmentation of their local and circulating concentrations is a new therapeutic strategy in CHF and atherosclerosis. Conversely ET-1 is a powerful vasoconstrictor, mitogenic and anti-natriuretic peptide which is produced in increased quantities in CHF (Love and McMurray 1996, Petrie et al 1999). ET-1 is also believed to contribute to the development and progression of atherosclerosis. Inhibition of the action of ET-1 may be beneficial in CHF and atherosclerosis (Love and McMurray 1997).

The NEP and ECE inhibitory properties of SLV 306 have been studied in animal models of acute and CHF, systemic and pulmonary hypertension. In all these models it has shown a very promising profile of activity. In animal studies a statistically significant diuretic and natriuretic activity was coupled with a dose dependent and statistically significant reduction in systemic systolic and diastolic blood pressure. In CHF models in animals SLV 306 improved cardiac performance, reduced lung oedema and cardiac remodelling and showed renal protective properties (Meil et al 1998).

To date, 97 healthy male and female subjects have been exposed to SLV 306 in four studies. In healthy subjects, SLV 306 has been well tolerated and considered safe in the doses given. Following administration of SLV 306 it is quickly absorbed and hydrolyzed to the active metabolite KC12615 (Meil and Wurl et al 1998). After single dosing, a dose dependent increase in plasma ANP and cGMP concentrations from about

**Figure 1.3.5**

The chemical structure of SLV 306 and its active metabolite KC 12615



50mg onwards has been observed; there is no demonstrable change in plasma ET-1 concentrations at this dose and no effect on cardiac output, mean arterial pressure or total peripheral resistance. After multiple dosing, ANP and cGMP increases after 200mg twice daily, while angiotensin II and renin decrease. Again there has been no change in plasma ET-1 concentrations demonstrated with this dosing regime (Meil and Rupp et al 1998). Clearly further investigation is required in order to fully establish the ECE inhibitory activity of SLV 306. Clear evidence of inhibition of the conversion of the precursor big ET-1 to the active vasoconstrictor peptide ET -1 is required. As part of this thesis I study further the pharmacologic actions of SLV 306.



## **1.4 ENDOTHELIAL FUNCTION**

### **– Vasomotor control**

The human vascular endothelium is not simply a semi permeable membrane allowing exchange of water and small molecules. It is also involved in vasomotor control, clotting homeostasis and regulation of vascular growth which are all key to cardiovascular health or disease. Vasomotor control has been adopted as the means by which endothelial function is assessed and certainly with regard to heart failure is often the focus of what is discussed when the term endothelial dysfunction is used.

Endothelial cells lie between the vascular lumen and the smooth muscle cells of the vessel wall. They sense mechanical forces within the lumen and regulate vascular tone through the production of a variety of factors (Kinlay et al 1996, Mombouli and Vanhoutte 1999). The endothelium produces both potent vasodilators, such as endothelium derived relaxing factor (EDRF), prostacyclin, and endothelium derived hyperpolarizing factor (EDHF) and vasoconstrictors such as ET-1 and angiotensin II. Local blood flow is thus determined by a balance between the effects of vasodilators and vasoconstrictors on the underlying smooth muscle.

In 1980 Furchgott demonstrated that acetylcholine had a vasodilatory effect on blood vessels in the presence of the endothelium but was associated with vasoconstriction if the endothelium was removed (Furchgott 1996). It became apparent that vasodilatation was mediated by the endothelium and vasoconstriction by the vascular smooth muscle. In healthy vessels in vivo the endothelium dependent vasodilatation predominates.

In 1986 Ignarro et al suggested that the mediator of endothelium dependent vasodilatation was the free radical NO (Ignarro et al 1987). NO appears to play a key role in many endothelial functions not least vasomotor control. NO is released as the product of a reaction between L-arginine and nitric oxide synthase (NOS) (Palmer et al 1988). It has a very short half life and therefore works on a very local or paracrine basis. It is important for maintenance of arterial relaxation at rest, its availability is improved by increased intraluminal flow, hypoxia (Brown et al 1993), bradykinin and other hormonal mediators. It acts by reducing the intracellular calcium concentration of vascular smooth muscle and thereby inducing relaxation of that smooth muscle or vasorelaxation.

Impaired endothelial function is said to contribute to the impaired vasodilator capacity seen in the vessels of patients with heart failure. This has been demonstrated by studies which report a reduced endothelium-dependent vasodilatation in response to infused acetylcholine and restoration of this response by administration of L-arginine, the precursor of endothelium derived NO (Hirooka et al 1994). The mechanisms potentially responsible include impaired endothelial cell receptor function, abnormal expression of NOS and the impaired release or rapid degradation of the EDRF - NO. The reduced blood flow and reduced shear stress as a result of reduced circulating volume in heart failure and increased ACE, angiotensin II and ET-1 activity could certainly be associated with such changes. The elevated plasma concentrations of cytokines in heart failure upregulate inducible NOS and increase basal NO release. Acetylcholine acts only on endothelial NOS, not inducible NOS, to increase NO release from the endothelium. Thus, although basal release of NO is increased, acetylcholine-stimulated vasodilatation is impaired. Perhaps the increase in basal release of NO is a compensatory response to impaired endothelial response. Unfortunately while impaired vasodilatation results in impaired exercise capacity, high concentrations of basal NO are directly cardiotoxic and can impair cardiac function, perhaps by increased apoptosis and reduced mitosis (Drexler 1999).

ET-1 is a potent vasoconstrictor released from the endothelium. It has a short half life like NO and is also involved in the maintenance of basal vascular tone (Haynes et al 1996). There are at least two distinct endothelin receptors; ET A and ET B. ET A receptors are found on the vascular smooth muscle cells and mediate vasoconstriction (Seo et al 1994). ET B receptors are found on both vascular smooth muscle cells, mediating vasoconstriction and endothelial cells. They mediate endothelial cell release of NO and prostacyclin to mediate vasodilatation (Tsukahara et al 1994). It is possible that the availability of NO plays a key role in the balance between ET A and ET B receptor activation and therefore vasomotor tone in response to ET-1 (Verhaar et al 1998).

Angiotensin II is another potent vasoconstrictor released by the endothelium as a result of the interaction of angiotensin I and ACE. The effect of angiotensin II on the endothelium, via AT<sub>1</sub> receptors is an increase in the production of superoxide anions which accelerate NO degradation and therefore reduces NO bioavailability (Griendling et al 1994). In contrast angiotensin II acts at AT<sub>2</sub> receptors to promote NO release from

endothelial cells (Wiemer et al 1993). In this regard selective AT<sub>1</sub> receptor antagonists may offer a benefit over ACE inhibition.

ACE is found in abundance in the vascular endothelium. Obviously it also contributes to vascular tone, not only through production of angiotensin II from angiotensin I resulting in vasoconstriction but also by an increase in the degradation of bradykinin (Pellacani et al 1994). Bradykinin acts on the endothelium, releasing NO and therefore causing vasodilatation. ACE inhibitors, by inhibiting bradykinin degradation upregulate NO activity. Angiotensin II receptor blockers do not reduce bradykinin breakdown and therefore cannot increase NO bioavailability via this mechanism. This is an advantage that ACE inhibitors have over angiotensin II receptor antagonists.

Overall it is suggested that ACE inhibitors and angiotensin II receptor antagonists improve endothelial function to a similar degree (Hornig et al 1997).

While it is suggested that the degree of endothelial dysfunction predicts survival and established treatments for heart failure such as ACE inhibitors, angiotensin II receptor antagonists, selected beta blockers and spironolactone are clearly associated with mortality benefit as well as improved endothelial function (Farquharson & Struthers 2000, Packer et al 1996, Pitt et al 1999) there is no direct evidence that improvement in endothelial function directly improves outcome. In fact there is some evidence to suggest that it does not. Endothelial dysfunction develops more rapidly in men than premenopausal women (Taddei et al 1996) and postmenopausal women have a rapid improvement in endothelial function following oestrogen therapy (Gilligan et al 1994) but hormone replacement therapy is not associated with improved outcome (Hulley et al 1998). Tumour necrosis factor (TNF) reduces the synthesis of NO and is associated with worsening endothelial function (Agnoletti et al 1999) but trials with etanercept, an anti TNF treatment, were not associated with improved outcome (Lisman et al 2002).

It is also unclear whether endothelial dysfunction has a causative role in the development of systolic myocardial dysfunction or is simply a marker of it.

Reduced effective circulating volume and therefore shear stress in heart failure means reduced production of NO. High levels of cytokines eg. TNF reduces the half life of NOS and therefore the synthesis of NO. Reduced bradykinin levels result in reduced NO production. The increase in oxygen free radicals as a result of activation of the

RAAS in heart failure accelerates NO degradation. Heart failure associated with myocardial ischaemia results in oxidative stress and an increase in oxygen free radicals which reduces NO bioavailability by these mechanisms. Overall heart failure reduces the availability of NO causing endothelial dysfunction.

However, coronary artery disease and hypertension, common causes of heart failure are in part the result of endothelial dysfunction. Risk factors for coronary artery disease have been associated with endothelial dysfunction. Endothelial dysfunction may allow access of neurohormones to vascular smooth muscle cells resulting in vasoconstriction rather than endothelial cell mediated vasodilatation. Endothelial dysfunction affects arterial compliance, increased arterial stiffness causes increased cardiac afterload and accelerates ventricular remodelling both resulting in systolic impairment.

The in vitro studies that form part of this thesis consider the vasoactive response of small resistance arteries to neurohormones and the effect of enzyme inhibition on this response. I hope to demonstrate the role of these enzymes in the production or degradation of each of the hormones. I am in effect measuring endothelial function in the presence of various neurohormones and their regulators.

## 1.5 Aims

During the development of now well established treatments, such as ACE inhibitors, we have learnt that inhibition of the activity of one vasoactive hormone often results in clinically important inhibition or potentiation of other pathways. Clearly there is value in antagonizing neurohumoral systems likely to have detrimental actions, and it is likely that the optimum strategy is to achieve this while simultaneously augmenting potentially desirable neurohumoral mediators. However enzyme inhibitors which act on multiple vasoactive mediators with complex interactions have unpredictable effects.

The work described in this thesis looks particularly but not exclusively at two recently developed molecules which have dual enzyme inhibitor activity. Omapatrilat, a molecule which inhibits both ACE and NEP, and SLV 306 (active metabolite KC12615) a compound with both NEP and ECE inhibiting properties. My intention is to further characterise these molecules and their therapeutic potential while utilising their inhibitory properties in further investigation of the human neurohumoral system.

1. To consider possible mediators of the unexpected and potentially life threatening side effect associated with omapatrilat; angioedema (chapter 3).
  - Of course bradykinin is a likely mediator, ACE and NEP are both involved in the metabolism of bradykinin. Also of interest is the role of NEP in metabolizing the vasodilator ADM. Other candidate vasodilators include CGRP which is of interest as a cause of facial flushing, substance P, and VIP.
2. To consider the possible mechanisms by which omapatrilat affects a more impressive hypotensive response in patients with hypertension than expected.
  - As ACE and probably NEP are both involved in the metabolism of bradykinin, this powerful vasodilator and others may play a role (chapter 3).
  - It has been suggested that the predominant physiological substrates for vascular NEP are vasoconstrictor peptides and particularly ET-1, explaining the contradictory haemodynamic results obtained after systemic dosing with NEP inhibitors. Inhibition of the vasoconstrictor response to angiotensin I or ET-1 by omapatrilat, another dual inhibitor of ACE / NEP, GW660511X and SLV 306

may demonstrate their advantage over the pure NEP inhibitor (chapters 4, 5 and 8).

3. To demonstrate the NEP and ECE inhibitory properties of SLV 306 in vitro - not previously demonstrated in human tissue either in vivo or in vitro (chapter 5).
4. To demonstrate the dose – response relationship for the ECE and NEP inhibitor activity of SLV 306 in healthy volunteers in vivo (chapter 7).
  - I evaluate the effect of the main metabolite of SLV 306 (KC12615) on the pressor response to big ET-1 and on neurohormones.
5. To consider the ACE and NEP inhibitory activity in vivo of the dual ACE/NEP inhibitor GW660511X (chapter 8).
  - a drug in early development which hopes to offer therapeutic benefit over that offered by pure ACE or NEP inhibitors but without the side effect profile of its forerunner omapatrilat.
  - ACE inhibitors being of proven benefit in cardiovascular disease remain the gold standard against which this new molecule must be compared and an active control is included in my experiment.
6. To demonstrate the haemodynamic and neurohumoral affects of renin inhibition in patients with chronic heart failure specifically a recently developed renin inhibitor aliskiren (chapter 9).

## 2 METHODS

### 2.1 PATIENTS AND HEALTHY VOLUNTEERS

The University of Glasgow is located adjacent to the Western Infirmary which is a tertiary health care centre. Patients attending cardiology clinic with a history of ischaemic heart disease but normal left ventricular systolic function were invited to take part in my studies. Their physiological responses were studied *ex vivo*, using wire myography. Patients taking warfarin therapy were excluded in order to avoid excessive bleeding during or following subcutaneous fat biopsy. Patients with renal dysfunction (creatinine > 200 μmol/l) were excluded.

Patients recruited for the *in vivo* study described in chapter 9 were required to meet the following criteria:

#### Inclusion criteria

- Male
- Aged 50-80 years
- LVEF  $\leq$  0.35
- Baseline treatment with ACE inhibitors and beta blockers stable for  $\geq$  1 month
- Stable NYHA II-IV

There were few exclusion criteria, mainly related to recent clinical instability (e.g. myocardial infarction), disorders that might impair absorption of therapy (e.g. pancreatic disease) or a contra-indication to ramipril (including renal artery stenosis).

The Clinical investigation and research unit is based at the Western Infirmary. A large number of healthy volunteer studies have been conducted at the centre and the staff were able to provide a database of volunteers who had previously, but not recently, taken part in studies. From this database and by word of mouth I recruited healthy volunteers for the *in vivo* studies described in chapters 6, 7, 8 and 10.

#### Inclusion criteria

- Male
- Age 18 – 45 years
- Body mass index (BMI) of >20 kg/m<sup>2</sup> and <26 kg/m<sup>2</sup>

- Caucasian subjects judged to be in good health based on the results of medical history, physical examination, laboratory profile and 12 lead ECG
- Heart rate between 45 and 100 bpm at rest.
- Systolic blood pressure  $\geq 100\text{mmHg}$  and  $\leq 140\text{mmHg}$  , diastolic blood pressure  $\geq 60\text{mmHg}$  and  $\leq 90\text{mmHg}$ .
- Written Informed consent given

### ***Exclusion criteria***

- Abnormal findings in medical history, physical examination, laboratory profile or 12 lead ECG
- Use of any prescribed and/or non-prescribed systemic or topically applied medication within 14 days or within 5 times the half-life of the respective drug, with the exception of paracetamol taken more than 48 hours prior to the start of the study.
- History of serious drug or allergic reaction
- Smoker
- Positive drug screen or suspicion of drug misuse (including alcohol)
- Participation in other clinical trials within the last three months
- Medical and nursing students

All patients and healthy volunteers provided written informed consent and all studies were approved by the West Ethics Committee of the Western Infirmary, on behalf of North Glasgow Hospitals University NHS Trust.

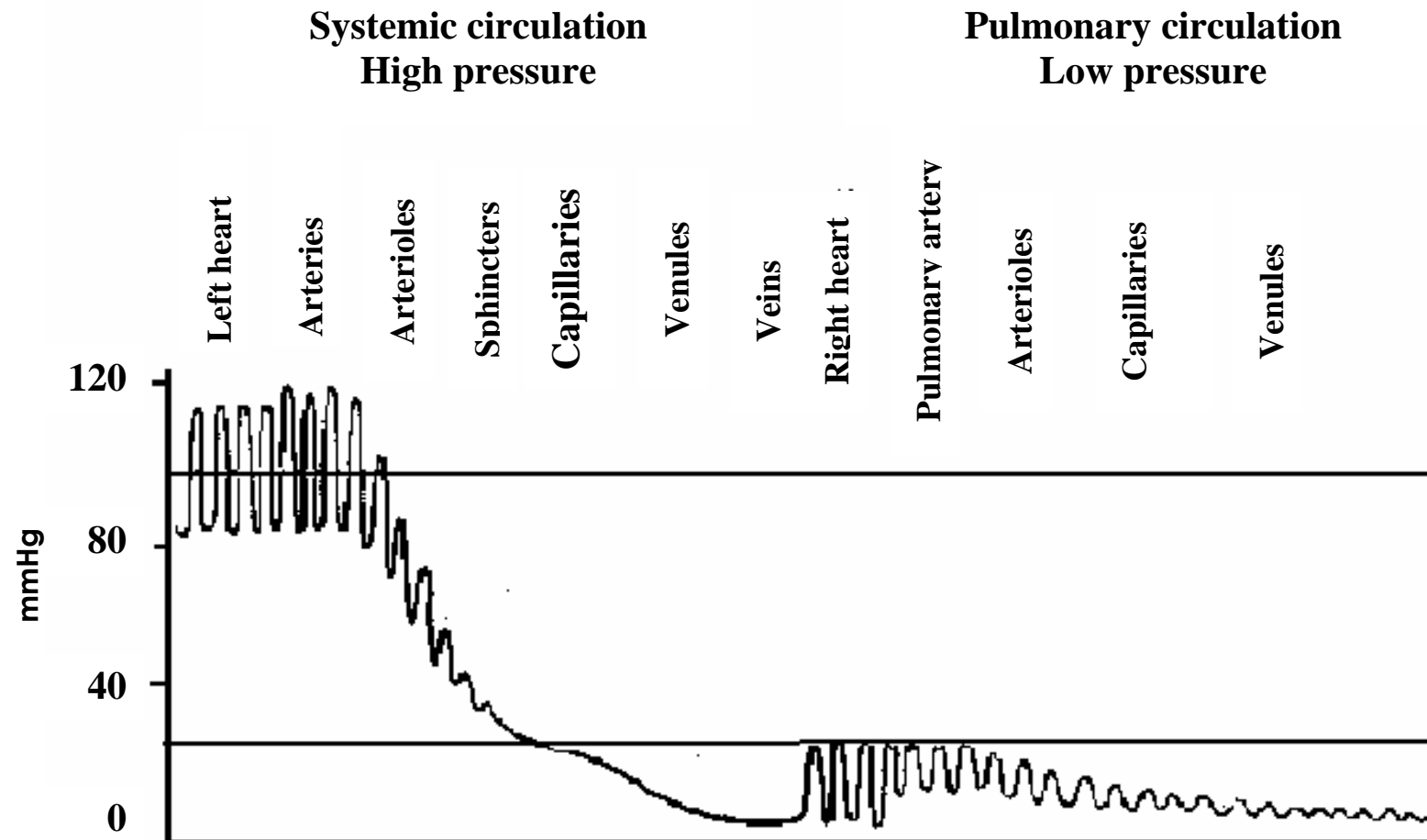
## **2.2 LEFT VENTRICULAR SYSTOLIC FUNCTION**

Transthoracic echocardiography was performed on all patients and healthy volunteers. Estimate of ejection fraction was made using Simpson's biplane method (Schiller et al 1979).



**Figure 2.1**

Small resistance arteries play a significant role in the regulation of vascular resistance



## **In vitro studies**

### SMALL RESISTANCE ARTERIES

Resistance arteries play a significant role in the regulation of vascular resistance and therefore blood flow and cardiac work load (Mulvany and Aalkjaer 1990) (figure 2.1). Small subcutaneous resistance arteries (SRA) of patients with normal left ventricular function were obtained by subcutaneous fat biopsy. Elliptical Subcutaneous gluteal biopsies were taken under aseptic conditions and local anaesthetic (1% lidocaine). The wound, approximately five centimetres in length was closed with absorbable suture to the deep layers and vicryl to the skin. The superficial suture was removed after seven days as long as the wound was found to be healing well on inspection. This method of obtaining SRA has been shown previously to provide arteries that are fully viable, demonstrated by a concentration dependent response to noradrenaline and angiotensin II with maximal force (Aalkjaer et al 1986)

Dissected tissue was then placed into cold 0.9% NaCl and transported immediately to the laboratory. There it was transferred to cold physiological salt solution [PSS](composition in mM: NaCl 118.4, KCl 4.7, MgSO<sub>4</sub>.H<sub>2</sub>O 1.2, KH<sub>2</sub>PO<sub>4</sub> 1.2, NaHCO<sub>3</sub> 24.9, CaCl<sub>2</sub> 2.5, glucose 11.1, EDTA 0.023 which gives a pH of 7.4 when gassed with a 5% CO<sub>2</sub>/95% O<sub>2</sub> mixture). Where possible, four SRA approximately 2mm in length, were isolated from each skin biopsy by careful dissection using surgical grade instruments and with the aid of a high power microscope. The dissection was performed in a Petri dish filled with the PSS buffer being changed regularly. The SRA were identified by their helical shape and opaque wall, in contrast to veins which are rather more elongated, flaccid and transparent. The dissection process could take anything up to two to three hours to complete. The dissected arteries were then stored in PSS at 4°C overnight. Storage of resistance arteries from rats in this way has been demonstrated to have no effect on their vasoactive properties (McIntyre et al 1998). It is now an established practice adopted by many groups reporting wire myography studies in both animal and human arteries.

### 2.4 WIRE MYOGRAPHY

SRA wire myography is an in vitro technique which allows resistance arteries with a diameter of 100-500µm to be studied under precise and standardised conditions. Use of

this technique allows the study of a vessel's contractile or relaxant properties under isometric tension (Mulvaney and Aalkjaer 1990, Mulvaney and Halpern 1977).

Approximately 24 hours after the initial biopsy, each vessel was mounted on to two 40µm diameter stainless steel wires, where one wire was attached to a force transducer and the other to a micrometer (figure 2.2). The temperature was raised to 37°C and a gas mixture (5% CO<sub>2</sub>/95% O<sub>2</sub>) was bubbled in for the duration of the experiment.

All my studies were undertaken in paired vessels from the same patient in every case. If clear results were not obtained from any single vessel, a record of no data was made. The vessel was not replaced.

After a rest period of one hour each artery was stretched at 1 minute intervals to determine the passive exponential wall tension-internal circumference (L) relationship. From the Laplace equation,  $P=T/r$  (where P is the effective pressure, T is the wall tension and r is the internal radius), the equivalent circumference (L<sub>100</sub>) for a transmural pressure of 100mmHg, was calculated for each vessel by an iterative computer method. Each vessel was then set to the normalised internal diameter,  $L_i=0.9 \times L_{100}\mu$ , at which contraction is thought to be optimal (Mulvaney and Aalkjaer 1990, Mulvaney and Halpern 1977, Petrie et al 2001).

After this normalisation procedure the arteries were exposed twice to high concentration potassium (KPSS is identical to PSS but with KCl substituted for NaCl on an equimolar basis) and once to 10µmol/L of norepinephrine (NE). After a plateau contraction had been attained with NE, 3µmol/L of ACh was added to the bath in order to stimulate endothelium dependent vasodilation. Arteries that were not seen to contract in response to either KPSS or NE or showed no relaxation to ACh were deemed to have no functionally intact endothelium and were therefore discarded.

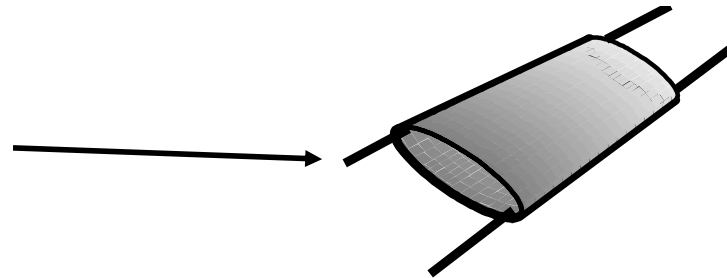
The arteries were then incubated for a further 30 minutes in PSS before being set up for study protocol.

In each of the studies PSS was then replaced by a solution of the enzyme inhibitor (table 2.1) to be studied or vehicle. Cumulative concentration response curves (CCRC) were

**Figure 2.2**

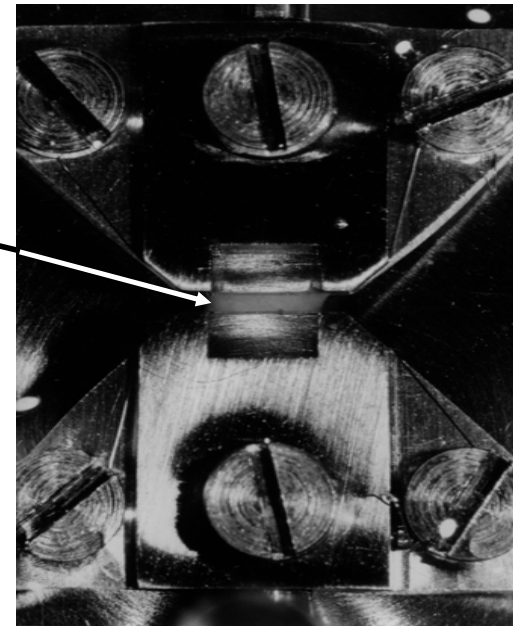
Wire myography. Each vessel is mounted on to two 40 $\mu$ m diameter stainless steel wires, where one wire is attached to a force transducer and the other to a micrometer (Mulvaney and Halpern 1976)

40  $\mu$ m tungsten wire



Vessel secured between two jaws

One jaw is fixed,  
the other is attached to  
a force transducer



<b>Name</b>	<b>Inhibitory properties</b>
Omapatrilat	ACE / NEP
KC12615	ECE / NEP
Captopril	ACE
Phosphoramidon	ECE / NEP
Thiorphan	NEP

**Table 2.1 Vasoactive enzyme inhibitors**

then obtained to various vasoactive hormones. For vasodilator curves, the concentration of agonist was increased as the relaxation response rate at each respective dose levelled off. If spontaneous relaxation back to baseline was seen, a common problem, the experiment was abandoned; the vessel washed with PSS and allowed to rest for 30 minutes, before a further attempt was made. For vasoconstrictor curves the concentration of hormone was increased after a minimum of five minutes, after contraction was seen and had peaked at each respective dose. At higher concentrations desensitisation or tachyphylaxis could be seen as the agonist became continually present at a receptor. After exposure to each hormone the vessels were washed with PSS again to re-establish baseline and inhibitors were again added to maintain the same relationship between inhibitor and vessel. After a further 30 minutes CCRC with other vasoactive hormones were obtained. Vessel relaxation in response to a hormone was reported as a percentage of the NE induced precontracted diameter of the artery. Vasoconstriction was expressed as a percentage of that elicited by the second exposure to KPSS.

### **In vivo studies**

## **2.6 INFUSIONS**

### **2.6.1 Big endothelin-1**

In order to assess the ECE inhibitory action of SLV 306 (chapter 7) I study the pressor response in healthy volunteers to big ET-1. The approach used is analogous to that used to study dose response relationships for ACE inhibitors (Biollaz et al 1981, Burnier et al 1981, Wellstein et al 1987). Both big ET-1 and ET-1 are endogenously synthesised peptides. Both have been infused systemically in healthy volunteers and in patients with cardiovascular disease previously, without any report of adverse event. The maximum increase in mean arterial pressure has been 33mmHg with the concentrations of big ET-1 used previously. The pressor response is known to develop slowly. From previous studies I had expected a peak increase in blood pressure after about 90 minutes from the start of the infusion, lasting for at least 90 minutes from completion of the infusion (Ahlborg et al 1994, Ahlborg et al 1996, Pernow et al 1996). In these previous studies a rising dose regime of 0.2, 1 and 8 pmol/kg/min, for 20 minutes each had been associated with a maximum increase in mean arterial pressure of 33mmHg. In my preliminary studies however this regime did not lead to a measurable increase in BP (BP monitored for 2 hours post infusion). The doses of big ET-1 used were therefore chosen from my pilot study (chapter 6), as ones likely to lead to a rise in mean arterial pressure of approximately 20 mmHg. The timing of the infusions was chosen to coincide with peak plasma concentrations of KC12615 as demonstrated in the pharmacokinetic first stage of my study (chapter 7). Subjects were given a systemic, intravenous infusion of big ET-1 160 minutes after dosing with placebo or SLV306. The infusion was commenced at a rate of 8.0pmol/kg/min. After 40 minutes (220-240 minutes post dosing) the infusion rate increased to 12pmol/kg/min and this rate of infusion continued for 20 minutes before being stopped. The big ET-1 was purchased from Clinalpha AG Laufelfingen, Switzerland.

#### 2.6.2 Angiotensin I

In order to assess the ACE inhibitory action of GW660511X (chapter 8) I study the pressor response in healthy volunteers to angiotensin I. The approach has been used previously to study dose response relationships for ACE inhibitors (Biollaz et al 1981, Burnier et al 1981, Wellstein et al 1987). Both angiotensin I and angiotensin II are endogenously synthesised peptides. Both have been infused systemically in healthy volunteers and in patients with cardiovascular disease previously, without any report of adverse event. Ascending dose infusion of angiotensin I (0.1, 0.3, 0.9, 2.0, 3.9, 6.0, 9.0,

12.0, 15.0 and 18.0 µg/min, each dose given for 3 minutes) was used, given 2, 4 and 23 hours after study medication was administered. The maximum increase in diastolic arterial pressure has been 25mmHg with the concentrations of angiotensin I when used previously. From these previous studies I expected a peak increase in blood pressure after about 3 minutes from the start of each dose of the infusion, lasting for approx. 1-3 minutes from completion of the infusion (Essig et al 1989, Erb et al 1991, Wellstein et al 1987) The doses were chosen as those shown to cause a haemodynamic response sufficient to show an influence on it by pharmacological intervention in these previous studies.

During screening the healthy volunteers received an angiotensin I infusion and only those with a rise in systolic blood pressure of at least 10mmHg were eligible.

The angiotensin I was purchased from Clinalpha AG, Laufelfingen, Switzerland.

## 2.7 BLOOD PRESSURE READINGS

Heart rate and arterial pressure were measured using an automated blood pressure recorder (Dinamap model 8262, 1846 SX Critikon inc., Tampa, Florida, USA). Measurements were taken from the contralateral arm to any infusion. Blood pressure measurements were made in duplicate on each occasion.

## 2.8 NEUROHORMONE ASSAYS

All blood samples taken during my studies were immediately placed on ice. They were then spun, as soon as possible in a refrigerated centrifuge (4°C). Plasma were spun at 1500g and then stored at -70°C in polypropylene tubes until assay.

In the SLV 306 studies, plasma big ET-1 and ET-1 levels were measured to assess the effect of SLV 306 and its metabolite on the conversion of big ET-1 to ET-1. Plasma ANP concentrations serve as a measure of the degree and time course of NEP inhibition.

Dr. A. Davenport, University of Cambridge kindly performed the ET-1 and big ET-1 assay by methods previously described (Plumpton et al 1995) and Dr. J.J. Morton, University of Glasgow kindly performed the ANP assays, again using methods previously described (McDonagh et al 1998).

In the GW660511X studies, blood samples were collected for measurement of plasma ACE activity, plasma ANP and plasma cyclic guanosine monophosphate (cGMP). Assays were performed by Dr. J.J. Morton, Glasgow University and Glaxo Smith Kline using methods previously described (McDonagh et al 1998, MacFadyen et al 1999, Nussberger et al 2002).

In the Aliskiren studies, plasma BNP, angiotensin II and aldosterone concentrations were kindly measured by Dr. J.J. Morton, Glasgow University, using established assays, as previously reported (Murdoch et al 1997, Harrap et al 1996, MacFadyen et al 1995). Plasma renin activity was also measured using a recognised assay (Nussberger et al 2002) by Dr. J. Nussberger, Lausanne, Switzerland.



### **3 Effects of Omapatrilat on the action of vasodilators in small human resistance arteries**

#### **Background**

Omapatrilat, the lead orally active dual NEP and ACE inhibitor, has a particularly marked hypotensive action. Studies have demonstrated greater effect on blood pressure in humans *in vivo* than were expected following animal studies (Campese et al 2001, Asmar et al 2000, Ferrario et al 2002, Mitchell et al 2002) or were predicted following studies with pure NEP inhibitors (Richards et al 1993, Favrat et al 1995). I studied the interaction of omapatrilat with vasoactive peptides. I included peptides not expected to be affected by its recognized pharmacological actions.

In addition to inhibition of angiotensin II production and inhibition of natriuretic peptide degradation, omapatrilat may inhibit the breakdown of other vasoactive peptides in human vessels (the pharmacology of which differs from other species). The studies described in this chapter focus specifically on the effects of omapatrilat on vasodilators while the subsequent chapter focuses on the effects of this inhibitor on vasoconstrictors. Augmentation of vasodilators particularly has been implicated in the increased incidence of angioedema seen in *in vivo* studies of omapatrilat.

Of particular interest is the role of NEP in metabolizing the newly discovered vasodilator ADM. Other candidate vasodilators include CGRP which is of particular interest as a cause of facial flushing (reported with omapatrilat) substance P, and VIP. As ACE and probably NEP are both involved in the metabolism of bradykinin, this powerful vasodilator may also play a role in the hypotensive action of omapatrilat. The vasodilator action of all of these peptides may be facilitated by the dual ACE/NEP inhibiting action of omapatrilat, compared to NEP inhibition monotherapy.

The aim of these studies is to fully characterize the vascular actions of omapatrilat in human vessels, *ex vivo*. These actions will be compared to those of an ACE inhibitor and those of a NEP inhibitor. This will reveal any "unrecognised" action of omapatrilat and/or synergy between NEP and ACE inhibition.

## Methods

Resistance arteries contribute the greatest resistance of all vessels to blood flow and therefore have a significant role in regulating capillary pressure (Mulvany and Aalkjaer 1990). I studied the effects of two concentrations of omapatrilat, reflecting the therapeutic range, on the vasopressor response of these vessels to various vasoactive peptides. For comparison, equipotent concentrations of an ACE inhibitor, captopril and a NEP inhibitor, thiorphan, were also studied.

SRAs were obtained from gluteal biopsies taken from patients with coronary artery disease but normal left ventricular systolic function not receiving ACE inhibitor therapy. Up to four vessels were dissected from each biopsy. These vessels were studied, *ex vivo*, in a wire myograph, according to the methods described in chapter 2. As part of the standard normalization and start up process the vessels were set to the internal diameter at which contraction is thought to be optimal, they were then exposed to a strong potassium solution (100mmol/l) repeatedly until maximal contraction was achieved. The endothelial viability of the arteries was assessed by precontraction with NE ( $10^{-6}$  mol/l) followed by relaxation with acetyl choline ( $10^{-6}$  mol/l).

The arteries were then incubated for 30 minutes in PSS prior to the construction of cumulative concentration response curves (CCRC) with various vasoactive peptides as detailed below. The studies were undertaken with paired vessels from the same patient in every case. Any vessels from which clear results were not obtained were recorded as lost data and not replaced other than with results from another complete set of paired vessels from a further patient.

### Myography protocols

1. Vessels were incubated for 30 minutes with vehicle (100% dimethyl sulfoxide [DMSO], vessel 1), captopril ( $10^{-5}$  mol/l, vessel 2), thiorphan ( $10^{-5}$  mol/l, vessel 3) or with omapatrilat ( $10^{-5}$  mol/l, vessel 4). The vessels were pre constricted with NE and CCRC were then obtained to CNP (dilatation). Responses to CNP are expressed as percent change to precontraction with  $10^{-5}$ M NE. This protocol was

performed in order to compare the effect of omapatrilat and the other inhibitors on NEP activity.

2. Vessels were incubated for 30 minutes with vehicle (100% dimethyl sulfoxide [DMSO], vessel 1), captopril ( $10^{-5}$  mol/l, vessel 2), thiorphan ( $10^{-5}$  mol/l, vessel 3) or with omapatrilat ( $10^{-5}$  mol/l, vessel 4). The vessels were pre-constricted with NE and CCRC (dilatation) were then obtained to bradykinin ( $10^{-14}$  –  $3 \times 10^{-6}$ M), ADM ( $10^{-11}$  –  $3 \times 10^{-7}$ M), CGRP ( $10^{-12}$  –  $3 \times 10^{-7}$ M), VIP and substance P. Responses to vasodilator are expressed as percent change to precontraction with NE. This protocol was performed in order to compare the effect of omapatrilat and the other inhibitors on important vasodilator activity

### Statistical analysis

Values are presented as mean  $\pm$  SEM. Statistical comparisons of  $pD_2$  (-log concentration required to produce 50% of the maximum response) and maximum response were performed with one-way analysis of variance (ANOVA) followed by Dunnet's post hoc test for multiple comparisons. The  $pEC^{50}$  was not determined where no maximum response was achieved at the concentrations used. Comparison of CCRC was by one-way ANOVA for repeated measures. Statistical significance was assumed at a value of  $p < 0.05$ .

## **Results**

### **Patient characteristics**

Biopsies were taken from 40 patients. The mean age of patients was  $64 \pm 10$  years and 27 were male. All had a diagnosis of ischaemic heart disease but preserved LV function with mean ejection fraction of  $51 \pm 8\%$ . 10 patients had coexistent hypertension. No patients were taking ACE inhibitors, other medication is detailed in Table 3.1

### **C-type natriuretic peptide CCRC: effect of captopril, thiorphan and omapatrilat.**

Compared to bradykinin, CNP was a weak vasodilator (maximal relaxation with vehicle  $52 \pm 13\%$  (n=8). The action of CNP was not augmented by any of the three enzyme inhibitors

**TABLE 3.1 Patient characteristics**

Patient characteristics	
Number of patients	40
Gender, M/F	27/13
Age, years	63.9 (10.4)
Previous MI, n	7
Previous CABG, n	6
Previous PTCA, n	6
Current smoker, n	9
Co existent hypertension, n	10
Mean ejection fraction, %	50.9(7.9)
Drug therapy, n	
ACE inhibitor	0
Diuretic	8
Digoxin	0
Calcium channel blocker	21
Oral nitrate	15
Beta-blocker	24
HMG-CoA reductase	23
Aspirin/clopidogrel	33
Glucose	5.6 (1.1)
Cholesterol	5.3 (1.1)
Creatinine	99.5 (16.0)
MI, myocardial infarction. CABG, coronary artery bypass grafting. PTCA, percutaneous transluminal coronary angioplasty. HMG CoA, 3-hydroxy-3-methylglutaryl coenzyme A. Values are given as number or mean (SD).	

(maximal responses:  $39 \pm 5\%$  captopril (n=6),  $37 \pm 19\%$  thiorphan (n=5)  $28 \pm 16\%$  omapatrilat (n=5).

**Bradykinin CCRC (figure 3.1): effect of captopril, thiorphan and omapatrilat.**

Bradykinin was a powerful vasodilator of precontracted small arteries and its vasorelaxant effect was significantly augmented by thiorphan, captopril and omapatrilat (figure 3.1,  $p < 0.01$  for comparison of curves) with a leftward shift of the concentration response curves. Captopril and omapatrilat produced almost a hundredfold shift ( $pD_2$ :  $8.95 \pm 0.09$  vehicle,  $10.62 \pm 0.09$  captopril,  $10.58 \pm 0.07$  omapatrilat) Whereas thiorphan produced only a ten-fold shift ( $pD_2$ :  $9.76 \pm 0.14$ ). The maximum responses with all 3 agents were, however, similar ( $94 \pm 1\%$  vehicle,  $93 \pm 3\%$  captopril,  $95 \pm 1\%$  thiorphan,  $93 \pm 2\%$  omapatrilat).

**Adrenomedullin CCRC (see figure 3.2): effect of captopril, thiorphan and omapatrilat.**

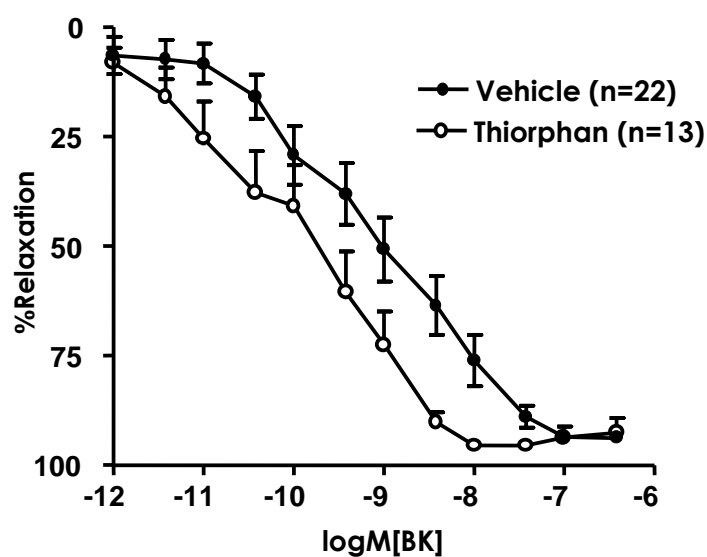
ADM was a more powerful vasodilator than CNP, but not as potent as BK. Neither captopril nor thiorphan augmented the vasodilator action of ADM, however, omapatrilat significantly potentiated the vasodilator response to ADM (figure 3.2,  $p < 0.05$ , comparison of curves). Omapatrilat resulted in a shift of the concentration response curve to the left, at lower concentrations of ADM. The maximal response however did not differ between groups ( $75 \pm 8\%$  vehicle,  $82 \pm 3\%$  captopril,  $68 \pm 9\%$  thiorphan,  $73 \pm 15\%$  omapatrilat).

**Calcitonin gene related peptide CCRC: effect of captopril, thiorphan and omapatrilat.**

None of the enzyme inhibitors affected the relaxation response to CGRP which was a potent vasodilator ((maximal responses:  $96 \pm 1\%$  vehicle (n=13),  $98 \pm 1\%$  captopril (n=4),  $88 \pm 6\%$  thiorphan (n=9),  $96 \pm 2\%$  omapatrilat (n=7);  $pD_2$ :  $9.6 \pm 0.1$  vehicle,  $9.8 \pm 0.1$  captopril,  $9.3 \pm 0.1$  thiorphan,  $9.8 \pm 0.1$  omapatrilat).

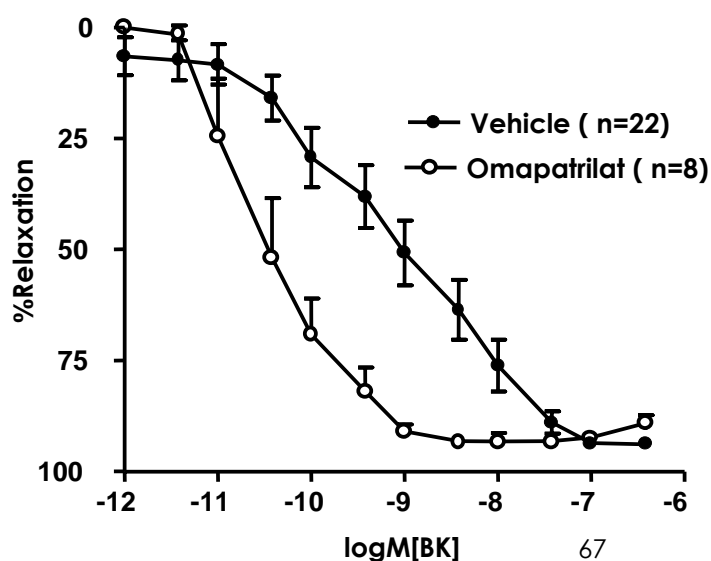
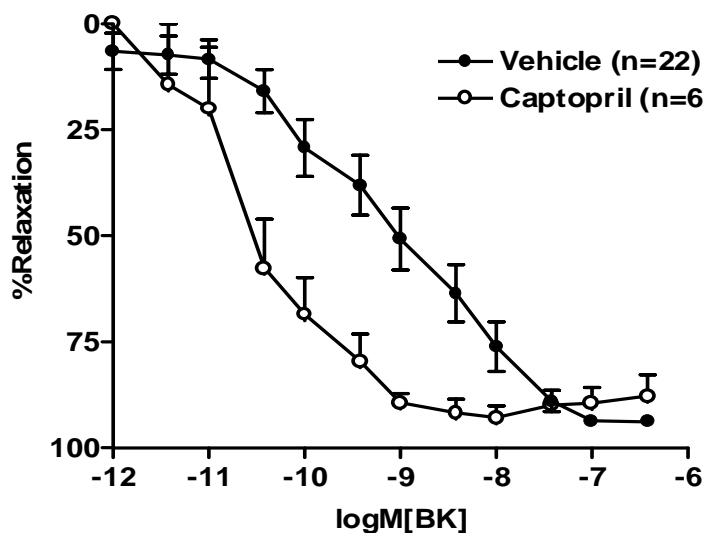
**Vasoactive intestinal polypeptide CCRC: effect of captopril, thiorphan and omapatrilat.**

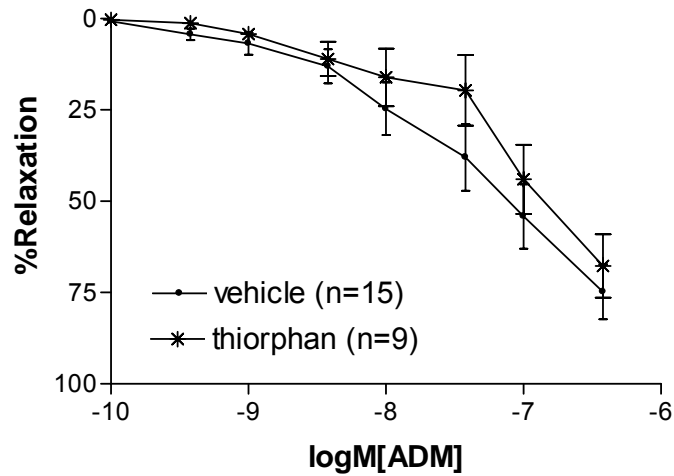
None of the enzyme inhibitors affected the relaxation response to VIP which was a moderately powerful vasodilator (maximal responses:  $65 \pm 9\%$  vehicle (n=14),  $78 \pm 6\%$  captopril (n=11),  $77 \pm 7\%$  thiorphan (n=8),  $70 \pm 8\%$  omapatrilat (n=12);  $pD_2$ :  $8.2 \pm 0.3$



**Figure 3.1**

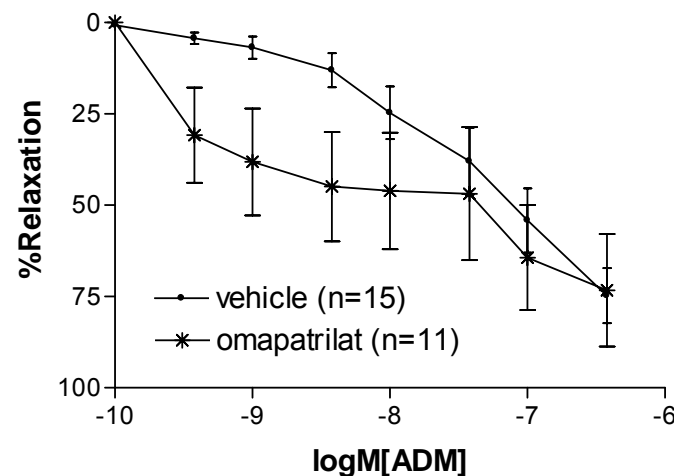
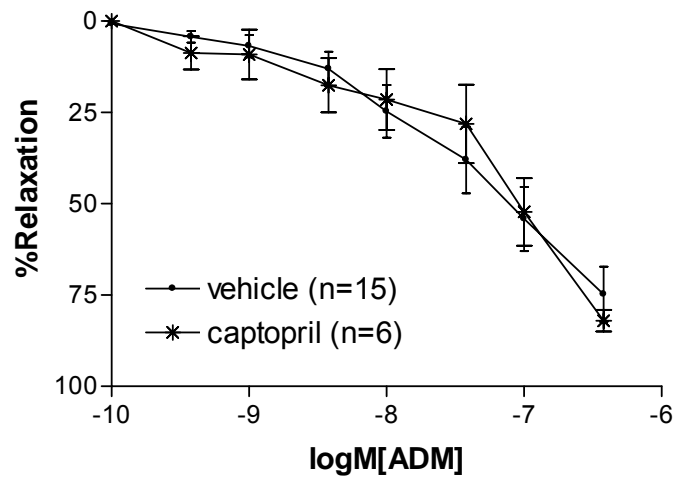
Cumulative concentration response (vasodilatation) curves for bradykinin (BK). Vasodilatation is expressed as percentage relaxation compared to the norepinephrine precontracted baseline. There was a powerful vasodilator effect in response to bradykinin which was significantly augmented by thiorphan, captopril and omapatrilat, with all three inhibitors producing a leftward shift of the concentration response curve ( $p < 0.01$ ).





**Figure 3.2**

Cumulative concentration response (vasodilatation) curves for adrenomedullin. Vasodilatation is expressed as percentage relaxation compared to the norepinephrine precontracted baseline. Neither thiorphan nor captopril augmented the vasodilator response to adrenomedullin. Omapatrilat, however, produced a significant augmentation, evident at lower concentrations only ( $p < 0.05$  by ANOVA).



vehicle,  $8.3 \pm 0.4$  thiorphan,  $8.8 \pm 0.1$  omapatrilat; a  $pD_2$  could not be calculated for captopril).

**Substance P CCRC: effect of captopril, thiorphan, omapatrilat and the combination of both thiorphan and captopril.** Substance P was the most potent of the vasodilators studies. Its action was not potentiated or inhibited by any of the enzyme inhibitors (maximal responses:  $90 \pm 2\%$  vehicle (n=6),  $90 \pm 2\%$  captopril (n=7),  $90 \pm 2\%$  thiorphan (n=5),  $85 \pm 5\%$  omapatrilat (n=7);  $pD_2$ :  $12.8 \pm 0.2$  vehicle,  $12.8 \pm 0.2$  captopril,  $12.6 \pm 1.1$  thiorphan,  $13.0 \pm 1.0$  omapatrilat).

## Discussion

Omapatrilat augmented the action of two important vasodilators in small resistance arteries from patients with coronary artery disease. Firstly, it markedly potentiated the vasorelaxation caused by bradykinin. NEP has been shown, experimentally, to metabolize bradykinin (Graf et al 1993, Blais et al 2000) and I have been able to confirm this in human arteries *ex-vivo*. However, thiorphan appeared to be less potent in this respect than captopril. Indeed, the range of captopril concentrations used in my study seemed to already maximally potentiate the effect of bradykinin (Graf et al 1993, Blais et al 2000). Consequently, omapatrilat did not enhance the vasodilator action of bradykinin more than captopril. This is not to say that the NEP inhibiting action of omapatrilat might not further inhibit bradykinin breakdown at lower ACE inhibitor concentrations or in other tissues, where greater amounts of NEP relative to ACE, may be found e.g. the myocardium (Kokkonen et al 1999, Corti et al 2001). This potential synergistic effect of ACE and NEP inhibition on bradykinin breakdown has been identified as a possible cause for concern, clinically, in relation to the risk of angio-oedema (Messerli and Nussberger 2000).

Another interesting and novel finding in the present study was the augmentation of ADM mediated vasorelaxation by omapatrilat. This has not been described previously though omapatrilat has recently been shown to increase plasma ADM concentrations in dogs with experimental heart failure (Cataliotti et al 2002). NEP inhibition is not the only explanation for my observations although perhaps the most obvious. Thiorphan did not have the same effect on the response to ADM as omapatrilat - either in the present



study or in a previous study (Messerli and Nussberger 2000). Others have, however, reported that an alternative NEP inhibitor, candoxatrilat, potentiates the renal actions of ADM in dogs (Lisy et al 1998). This apparent difference may have arisen because the kidney is particularly rich in NEP or because thiorphan is a weaker NEP inhibitor than omapatrilat. I favour the latter explanation because another potent NEP inhibitor, SCH 32615, increases plasma concentrations of ADM in sheep (Rademaker et al 2002).

Regardless of its mechanism, this additional action may certainly go some way towards explaining the unusual antihypertensive efficacy of omapatrilat. As has been reported, and confirmed in this study, ADM is one of the most effective endogenous vasodilators recognized, being substantially more potent than CNP and VIP (Lainchbury et al 1997, Jougasaki and Burnett 2000, Troughton and Lewis et al 2000). Consequently, the augmenting action of omapatrilat on the arterial action of this peptide could lead to an additional hypotensive effect, incremental to that expected from inhibition of angiotensin II production and natriuretic peptide breakdown.

As well as having a hypotensive effect, ADM may also protect the cardiovascular system against a number of noxious stimuli (Shimosawa et al 2002). It may also be important in other cardiovascular disease states. For example, ADM production is increased in CHF and ADM may have beneficial actions in this condition (Cataliotti et al 2002, Nagaya et al 2000, Jougasaki et al 1996). It has also been suggested that the pleiotropic actions of ADM are important in atherosclerosis (Nakayama et al 1999). Consequently, the augmenting effect of omapatrilat on the action of ADM could be therapeutically relevant in a number of cardiovascular conditions.

Interestingly, however, the activity of CGRP, which has some structural homology with ADM, was not enhanced by omapatrilat.

Similarly, omapatrilat did not augment the vasorelaxant action of CNP. At first sight, this might seem a curious finding given the role of NEP in natriuretic peptide catabolism. Others, however, have also reported that acute NEP inhibition does not potentiate the cardiovascular actions of exogenous CNP (Brandt et al 1997). Brandt et al found that, when co-infused with CNP in dogs, candoxatrilat increased circulating CNP concentrations. However, the haemodynamic (and renal) actions of exogenous CNP were not enhanced by candoxatrilat (Brandt et al 1997). Of course, my findings do

not preclude effects of NEP inhibition mediated by decreased clearance of ANP and BNP. Finally omapatrilat had no effect on the action of VIP or substance P in small resistance arteries.

In summary, omapatrilat augmented the action of bradykinin and, unexpectedly, the action of ADM in small human resistance arteries. This range of actions may explain the powerful hypotensive effect of omapatrilat *in vivo*, it may go some way to explaining the potentially life threatening side effect of angioedema associated with omapatrilat and it suggests additional therapeutic applications, for example in heart failure and atherosclerosis.

## **4 Effects of omapatrilat on the action of vasoconstrictors in small human resistance arteries**

### **Background**

The hypotensive effects of omapatrilat, a combined ACE and NEP inhibitor, cannot be explained by inhibition of angiotensin II production or inhibition of natriuretic peptide degradation alone. I have considered the effects of dual inhibition on the actions of vasodilators. The following group of studies aims to characterise the vascular actions of omapatrilat in human vessels, *ex vivo*, but concentrating on its effect on the potent vasoconstrictors angiotensin II and ET-1. These actions are compared with those of an ACE inhibitor and those of a NEP inhibitor in order to screen for any "unrecognised" action of omapatrilat and/or synergy between NEP and ACE inhibition.

A synergistic interaction between ACE and NEP inhibition is of interest because of the possible role of NEP in metabolising angiotensin II. Reduced breakdown of angiotensin II could offset the hypotensive action of augmenting plasma natriuretic peptide concentrations and, at least in part, account for the disappointing anti-hypertensive action of NEP inhibitors. Concomitant ACE inhibition should, of course, reduce the contribution of angiotensin II and allow full expression of the blood pressure lowering actions of natriuretic peptides.

Omapatrilat may inhibit another important metalloprotease in human vessels, namely ECE. Inhibition of the production of the potent vasoconstrictor ET-1 would be expected to further enhance the blood pressure lowering effect of a dual ACE/NEP inhibitor.

### **Methods**

I studied the effects of two concentrations of omapatrilat, reflecting the therapeutic range, on the vasopressor response of SRA to various vasoactive peptides. For comparison, equipotent concentrations of an ACE inhibitor, captopril and a NEP inhibitor, thiorphan, were also studied. In some studies the dual NEP-ECE inhibitor phosphoramidon was also used. Phosphoramidon is a well characterized inhibitor of NEP and ECE (Petrie, Hillier et al 2001, Jeng et al 1989, Ikegawa et al 1990).

SRA were obtained from gluteal biopsies taken from patients with coronary artery disease but normal left ventricular systolic function not receiving ACE inhibitor therapy. Up to four vessels were dissected from each biopsy. These vessels were studied, *ex vivo*, in a wire myograph, according to the methods described in chapter 2. As part of the standard normalization and start up process the vessels were set to the internal diameter at which contraction is thought to be optimal, they were then exposed to a strong potassium solution (100mmol/l) repeatedly until maximal contraction was achieved. The endothelial viability of the arteries was assessed by preconstriction with NE ( $10^{-6}$  mol/l) followed by relaxation with acetyl choline ( $10^{-6}$  mol/l).

The arteries were then incubated for 30 minutes in PSS prior to the construction of CCRC with various vasoactive peptides as detailed below. The studies were undertaken with paired vessels from the same patient in every case. Any vessels from which clear results were not obtained were recorded as lost data and not replaced other than with results from another complete set of paired vessels from a further patient.

#### Myography protocols

1. Vessels were incubated for 30 minutes with vehicle (dimethyl sulfoxide [DMSO], vessel 1), captopril (both  $10^{-5}$  mol/l, vessel 2), thiorphan ( $10^{-5}$  mol/l, vessel 3) or omapatrilat ( $10^{-5}$  mol/l, vessel 4). CCRC were then obtained to angiotensin I ( $10^{-11}$  -  $3 \times 10^{-6}$  mol/l, constriction). This protocol was performed in order to compare the effect of omapatrilat and the other inhibitors on ACE activity.
2. Vessels were incubated for 30 minutes with vehicle (100% dimethyl sulfoxide [DMSO], vessel 1), phosphoramidon ( $10^{-5}$  mol/l, vessel 2), captopril ( $10^{-5}$  mol/l, vessel 3) or omapatrilat ( $10^{-5}$  mol/l, vessel 4). CCRC were then obtained to big ET-1 ( $10^{-11}$  -  $10^{-6}$  mol/l, constriction). This protocol was performed in order to compare the effect of omapatrilat and the other inhibitors on ECE activity.

#### Statistical analysis

Values are presented as mean  $\pm$  SEM. Statistical comparisons of  $pD_2$  (-log concentration required to produce 50% of the maximum response) and maximum

response were performed with one-way ANOVA followed by Dunnet's post hoc test for multiple comparisons. The  $pEC^{50}$  was not determined where no maximum response was achieved at the concentrations used. Comparison of CCRC was by one-way ANOVA for repeated measures. Statistical significance was assumed at a value of  $p < 0.05$ .

## Results

### Patient characteristics

Further small resistance arteries were dissected from the same biopsies as those taken for the studies described in chapter 3. Patient characteristics are therefore as described in chapter 3 (Table 3.1).

**Angiotensin I CCRC (see figure 4.1):** Characteristic bell shaped concentration response curves were obtained with angiotensin I. As expected, the vasoconstrictor response was blocked by captopril (comparison of curves:  $p < 0.05$ ). Omapatrilat had a similar inhibitory action (comparison of curves:  $p < 0.05$ ) whereas thiorphan did not block the vasoconstrictor effect of angiotensin I. Indeed, there was a trend for enhanced vasoconstriction with thiorphan. The maximum responses were  $27 \pm 8\%$  vehicle,  $6 \pm 2\%$  captopril,  $39 \pm 10\%$  thiorphan,  $8 \pm 7\%$  omapatrilat; The  $pD_2$  concentrations were:  $8.0 \pm 0.3$  vehicle,  $8.4 \pm 0.2$  captopril,  $8.0 \pm 0.2$  thiorphan,  $7.5 \pm 0.3$  omapatrilat.

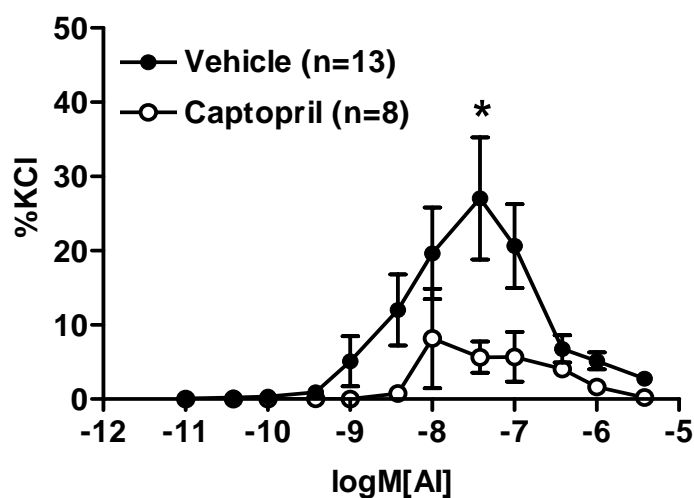
**Big endothelin-1 CCRC (see figure 4.2):** As previously described, big ET-1 had a much more powerful vasoconstrictor action than angiotensin I and, as expected, this was significantly attenuated by phosphoramidon which shifted the concentration response curve to the right.(comparison of curves:  $p < 0.05$ ). Neither captopril nor omapatrilat had any effect on big ET-1 induced vasoconstriction. The maximal responses with vehicle, phosphoramidon, captopril and omapatrilat were  $108 \pm 14$ ,  $101 \pm 18$ ,  $80 \pm 13$  and  $95 \pm 9\%$ , respectively.

## Discussion

Omapatrilat, as expected, attenuated the vasoconstrictor response to angiotensin I in small human resistance arteries. Interestingly, thiorphan, which only inhibits NEP, tended to enhance the vasoconstrictor response to angiotensin I (figure 4.1). This is in keeping with previous evidence that NEP may be involved in the degradation of

angiotensin II (Richards et al 1992) and underscores the synergistic basis of dual NEP-ACE inhibition. Omapatrilat did not affect the response to big ET-1 i.e. it did not inhibit ECE.

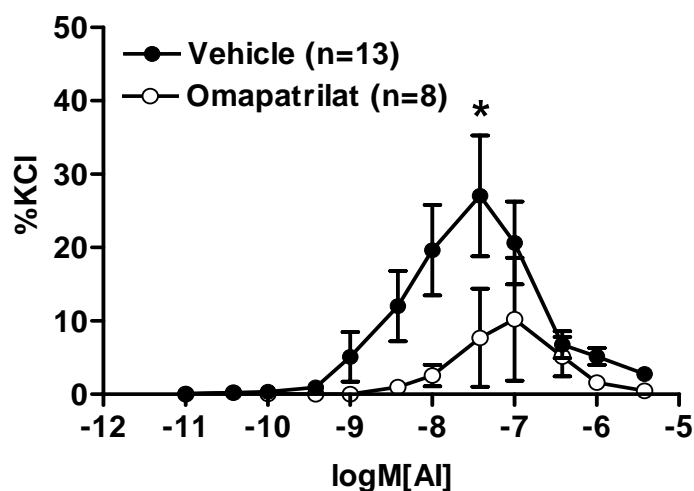
(a)



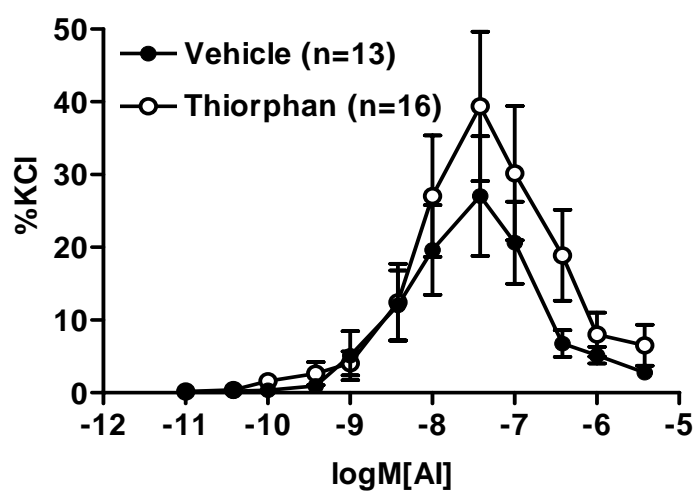
**Figure 4.1**

Cumulative concentration response (vasoconstriction) curves for angiotensin I. Vasoconstriction is expressed as a percentage of that obtained with potassium chloride KCl. There was a typical bell shaped vasoconstriction response. Captopril and omapatrilat, but not thiorphan, blocked the response to angiotensin I (both  $p < 0.05$  by ANOVA).

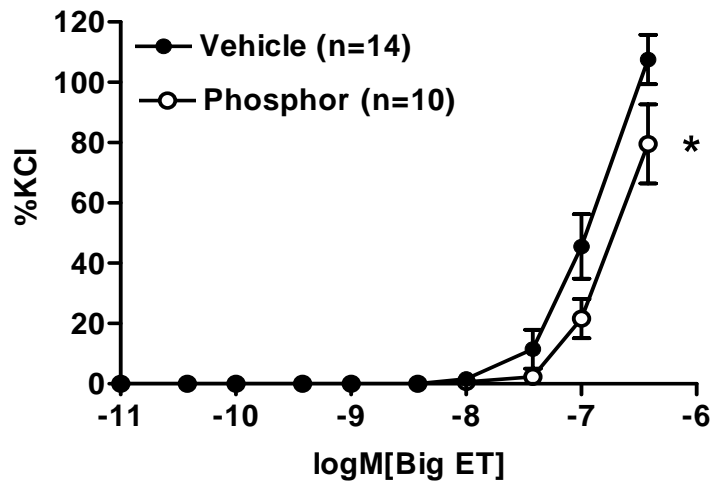
(b)



(c)



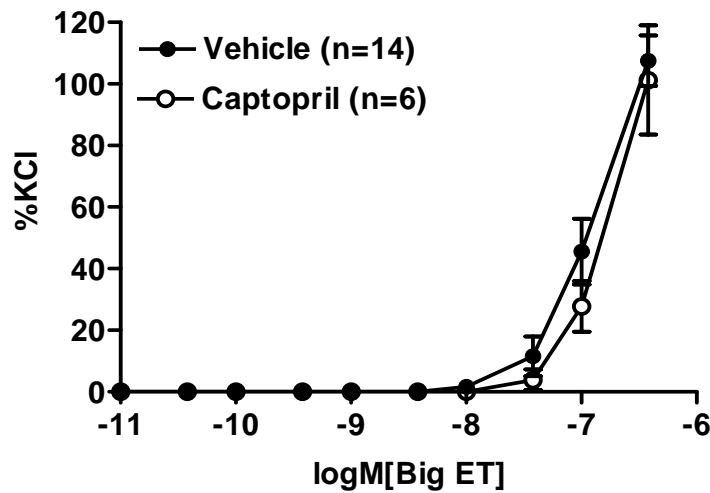
(a)



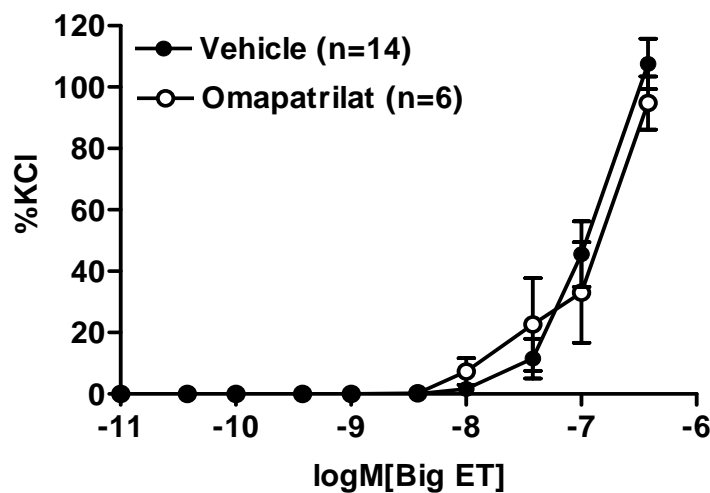
**Figure 4.2**

Cumulative concentration response (vasoconstriction) curves for big endothelin-1. There was a powerful vasoconstrictor response with no true maximum attained within the range studied. Phosphoramidon blocked big endothelin-1 dependent vasoconstriction ( $p<0.05$ ) whereas captopril and omapatrilat had no effect.

(b)



(c)





## **5 Characterisation of the human vascular actions of a novel dual inhibitor of neutral endopeptidase and endothelin converting enzyme in human small resistance arteries**

### **Background**

A pathophysiological role for both the natriuretic peptides and ET-1 has been postulated in hypertension, heart failure and atherosclerosis (Burnett 1989, Lerman et al 1991, Love and McMurray 1997, Schirger et al 2000). Broadly, the natriuretic peptides are considered to exert beneficial effects in these cardiovascular diseases whereas ET-1 is thought to have adverse actions. Consequently, augmentation of the effects of natriuretic peptides and inhibition of the actions of ET-1 are each considered desirable therapeutic strategies (Krum et al 1998, Northridge et al 1999, Best and Lerman 2000). Early clinical experience with NEP inhibitors, developed to block the breakdown of natriuretic peptides was, however, disappointing (Bevan et al 1992, Francis 1999). NEP inhibitors were ineffective anti-hypertensive agents (Bevan et al 1992). This was probably because NEP also catabolises angiotensin II and possibly ET-1, emphasizing the important interactions between these cardiovascular peptides (Richards et al 1992, Richards et al 1993, Russell et al 1996). A novel pharmacologic approach, taking advantage of this interaction and offering therapeutic synergy, is dual inhibition of NEP and ECE. I describe a series of experiments undertaken to characterise the actions of a new NEP-ECE inhibitor, KC 12615, in human blood vessels (Meil, Wurl et al 1998, Hillier, Berry et al 2001). While KC 12615 does appear to have NEP and ECE inhibitory actions in vitro, in tissues from experimental animals, they have not previously been demonstrated in human tissue in vitro or in vivo.

### **Methods**

SRA were obtained from gluteal biopsies taken from patients with coronary artery disease but normal left ventricular systolic function. Regular medication, particularly the use or not of ACE inhibitors was noted.

These vessels were studied, *ex vivo*, in a wire myograph, according to the methods described in chapter 2. After a standard normalisation and start-up protocol, involving

repeated washes with high potassium solution (60mM), the endothelial viability of the arteries was assessed by precontraction with NE ( $10^{-6}$ M) followed by relaxation with acetyl choline ( $10^{-6}$ M).

The arteries were then incubated for 30 minutes in PSS prior to the construction of CCRC with various vasoactive peptides as detailed below. The studies were undertaken with paired vessels from the same patient in every case. Any vessels from which clear results were not obtained were recorded as lost data and not replaced other than with results from another complete set of paired vessels from a further patient.

#### Vasoconstrictor protocols

After 30 minutes incubation of arteries with vehicle (PSS), phosphoramidon, (a dual NEP and ECE inhibitor) or KC 12615 (at  $10^{-4}$  M), CCRCs (vasoconstriction) were constructed with the ET-1 precursor, big ET-1 over the concentration range of  $10^{-10}$  to  $3 \times 10^{-7}$  M.

#### Vasodilator protocols

Again after 30 minutes incubation of arteries with vehicle (PSS), phosphoramidon, thiorphan or KC 12615 (at  $10^{-4}$  M), CCRCs were constructed with the following vasodilators in vessels pre-constricted with  $10^{-5}$ M norepinephrine: ANP ( $10^{-14}$  -  $3 \times 10^{-7}$ M ), bradykinin ( $10^{-14}$  -  $3 \times 10^{-6}$ M), ADM ( $10^{-11}$  -  $3 \times 10^{-7}$ M) and CGRP ( $10^{-12}$  -  $3 \times 10^{-7}$ M).

#### Statistical analysis

Vasoconstriction data are presented as a percentage of the contraction achieved with 60 mM potassium solution. Those for relaxation are represented as percent relaxation relative to the precontracted baseline. Values are presented as mean  $\pm$  SEM. Statistical comparisons of pD<sub>2</sub> (-log concentration required to produce 50% of the maximum response) and maximum response were performed with one-way ANOVA followed by Dunnet's post hoc test for multiple comparisons. Where no maximum response was achieved at the concentrations used, the pD<sub>2</sub> could not be determined and is not reported.

Comparison of CCRCs was by one-way ANOVA for repeated measures. Statistical significance was assumed at a value of  $p < 0.05$ .

Because prior treatment with an angiotensin converting enzyme (ACE) inhibitor was expected to affect the action of bradykinin, patients were split into an ACE inhibitor pre-treated and ACE inhibitor untreated group to analyse the response to bradykinin (prior treatment with an ACE inhibitor did not influence the response to other vasodilators).

## RESULTS

### Patient characteristics

Biopsies were taken from 49 patients. All had a diagnosis of ischaemic heart disease but preserved LV function. Medication is detailed in Table 5.1. Of note the group of patients taking ACE inhibitors is otherwise well matched to the group of patients not taking ACE inhibitors.

### Vasoconstrictor protocols

**Big endothelin-1** (figure 5.1): As previously described, big ET-1 had a powerful vasoconstrictor action. As expected, this was significantly attenuated by phosphoramidon (mean difference vs vehicle 12.05%,  $p < 0.01$ , 95% CI 2.767 to 21.34). KC 12615 ( $10^{-4}$  M), however, had the greatest inhibitory effect on big ET-1 induced vasoconstriction (mean difference vs vehicle 17.75%,  $p < 0.01$ , 95% CI 1.940 to 33.57 both by ANOVA for repeated measures).

### Vasodilator protocols

**Bradykinin** (figure 5.3): Bradykinin was a powerful vasodilator of precontracted small arteries, producing similarly powerful maximum vasodilator responses in all groups (maximal response:  $94 \pm 1\%$  vehicle,  $95 \pm 2\%$  phosphoramidon,  $94 \pm 2\%$  thiorphan,  $97 \pm 1\%$  KC12615). However, a leftwards shift in sensitivity was evident with all three inhibitors ( $p < 0.01$ , comparison of curves). This was also reflected in a reduced concentration of each inhibitor required to produce a 50% response ( $pEC_{50}$ :  $9.02 \pm 0.06$  logM vehicle,  $9.65 \pm 0.08$  logM phosphoramidon,  $9.95 \pm 0.16$  logM thiorphan,  $9.58 \pm 0.15$

**Table 5.1 Patient characteristics**

<b>Patient characteristics</b>	<b>Treatment includes ACE inhibitor</b>	<b>Treatment does not include ACE inhibitor</b>
Number of patients	22	27
Gender, M	13	16
Age, years	57.0 (16.1)	60.3 (11.5)
Previous MI, n	7	7
Previous CABG, n	6	5
Previous PTCA, n	5	7
Current smoker, n	4	8
Co existant hypertension, n	7	7
Co existent T2DM, n	1	1
Mean ejection fraction, %	53.6 (8.7)	51.6 (8.0)
Drug therapy, n		
ACE inhibitor	22	0
Diuretic	9	4
Digoxin	0	0
Calcium channel blocker	7	9
Oral nitrate	5	8
Beta-blocker	17	16
HMG-CoA reductase	16	12
Aspirin/clopidogrel	18	21
Glucose	6.5 (2.2)	6.4 (2.9)
Cholesterol	4.9 (0.7)	5.2 (0.7)
Creatinine	105.5 (16.4)	104.4 (25.4)
MI,myocardial infarction. CABG, coronary artery bypass grafting. PTCA, percutaneous transluminal coronary angioplasty. T2DM Type II diabetes mellitus. HMG CoA, 3-hydroxy-3-methylglutaryl coenzyme A. Values are given as number or mean (SD).		

logM KC12615), although this effect only achieved statistical significance with KC12615 ( $p < 0.05$ , figure 3).

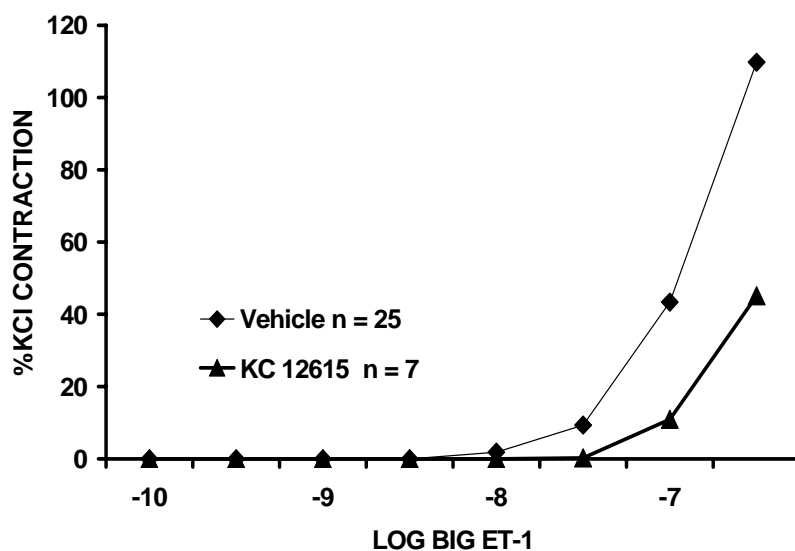
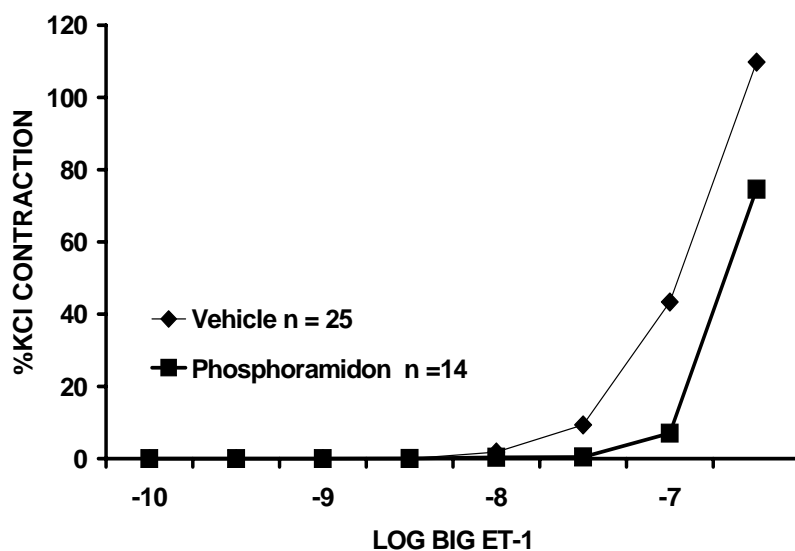
When patients were separated into those taking and those not taking an ACE inhibitor, KC 12615 was found to significantly augment bradykinin mediated vasodilatation only in the absence of an ACE inhibitor (pEC<sub>50</sub>:  $8.66 \pm 0.15$  logM vehicle,  $10.68 \pm 0.38$  logM KC12615,  $p < 0.05$ ). In patients pre-treated with an ACE inhibitor, the pEC<sub>50</sub> was  $8.75 \pm 0.10$  logM for vehicle and  $9.18 \pm 0.04$  logM for KC12615 (figure 5.4). This suggests that the effect of bradykinin is maximally augmented by ACE inhibition and that NEP inhibition does not lead to further augmentation.

**Adrenomedullin** (figure 5.5): ADM was also a potent vasodilator. As with ANP, the response to ADM was enhanced after incubation with both dual enzyme inhibitors ( $p < 0.05$ , comparison of curves) but not with thiorphan. In contrast to ANP, however, no difference was observed in the maximal response (maximal response:  $78 \pm 6\%$  vehicle,  $79 \pm 8\%$  phosphoramidon,  $74 \pm 7\%$  thiorphan,  $75 \pm 8\%$  KC12615)

**Calcitonin gene related peptide** (figure 5.6): Phosphoramidon significantly augmented the response to CGRP ( $p < 0.05$ , comparison of curves). This effect was most marked at the low concentrations of CGRP. However neither the maximal response ( $96 \pm 1\%$  vehicle,  $98 \pm 1\%$  phosphoramidon,  $93 \pm 4\%$  thiorphan,  $97 \pm 2\%$  KC12615) nor the pEC<sub>50</sub> ( $9.54 \pm 0.08$  logM vehicle,  $10.05 \pm 0.24$  logM phosphoramidon,  $9.52 \pm 0.04$  logM thiorphan,  $9.55 \pm 0.11$  logM KC12615), were significantly different. The other inhibitors had no effect on the response to CGRP.

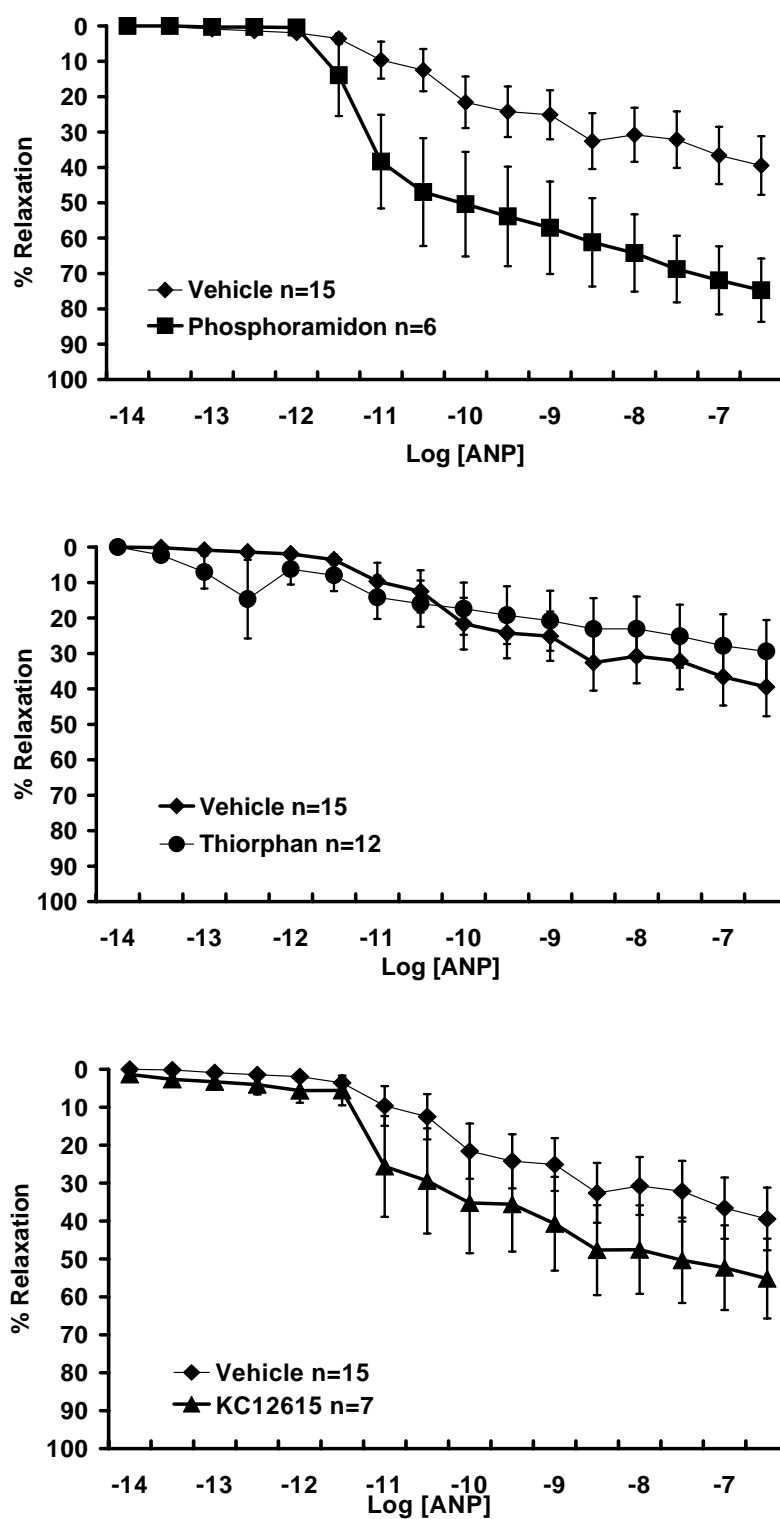
**Figure 5.1**

Cumulative concentration response curves (vasoconstriction) for big endothelin-1 (big ET-1). Vasoconstriction is expressed as a percentage of that obtained with potassium chloride, (KCl). Big endothelin-1 has a powerful vasoconstrictor action. The curves are shifted to the right in the presence of  $10^{-4}$ M phosphoramidon and  $10^{-4}$ M KC 12615. The vasoconstrictor effect of big endothelin-1 is significantly attenuated by phosphoramidon ( $p < 0.01$ ) and more markedly by KC12615 ( $p < 0.01$  by ANOVA for repeated measures).



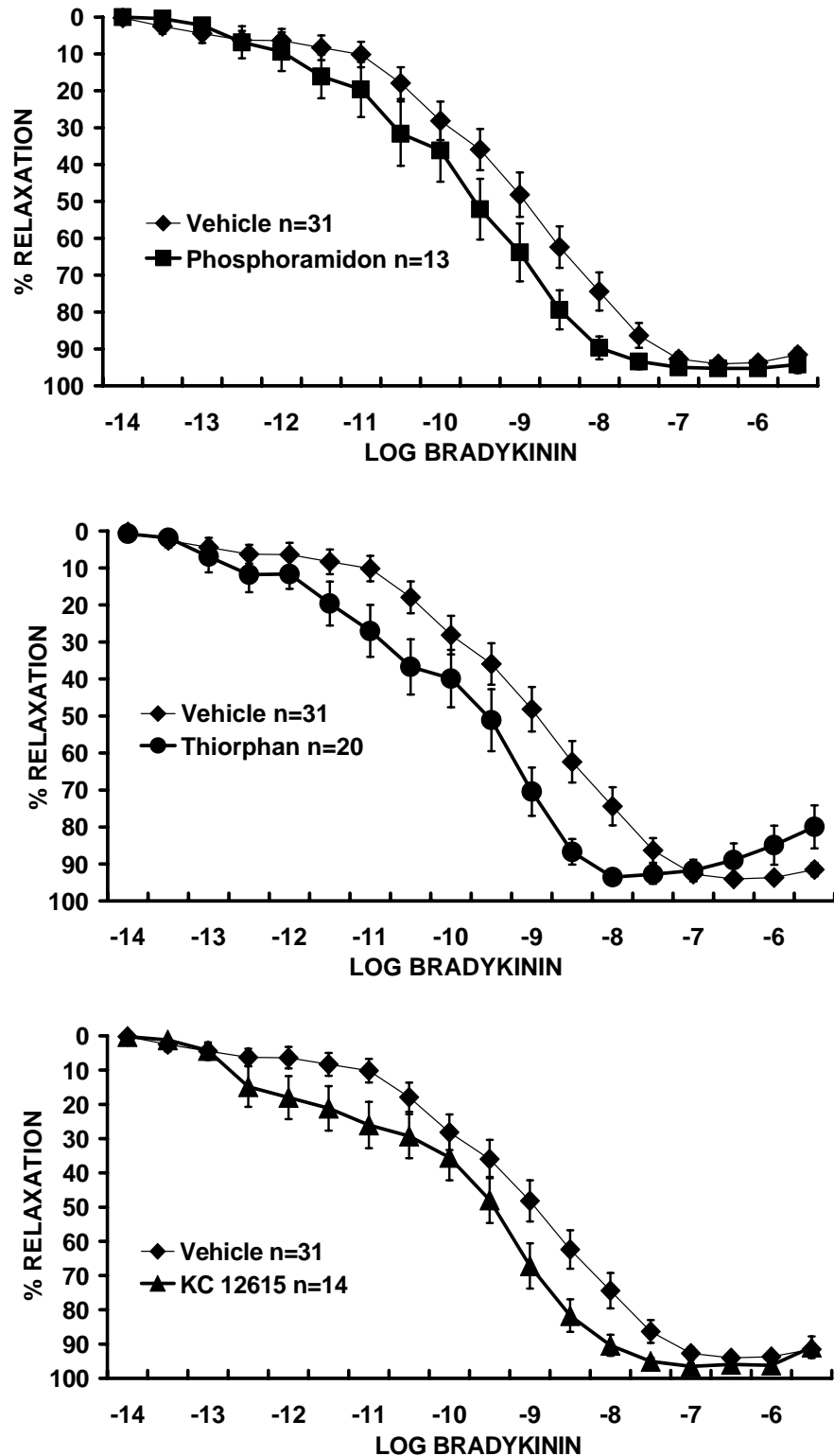
**Figure 5.2**

Cumulative concentration response curves (vasodilatation) for atrial natriuretic peptide (ANP). Atrial natriuretic peptide is a relatively weak vasodilator. Its effects are augmented by phosphoramidon and KC12615 (both  $p < 0.05$ ), but not by thiorphan.



**Figure 5.3**

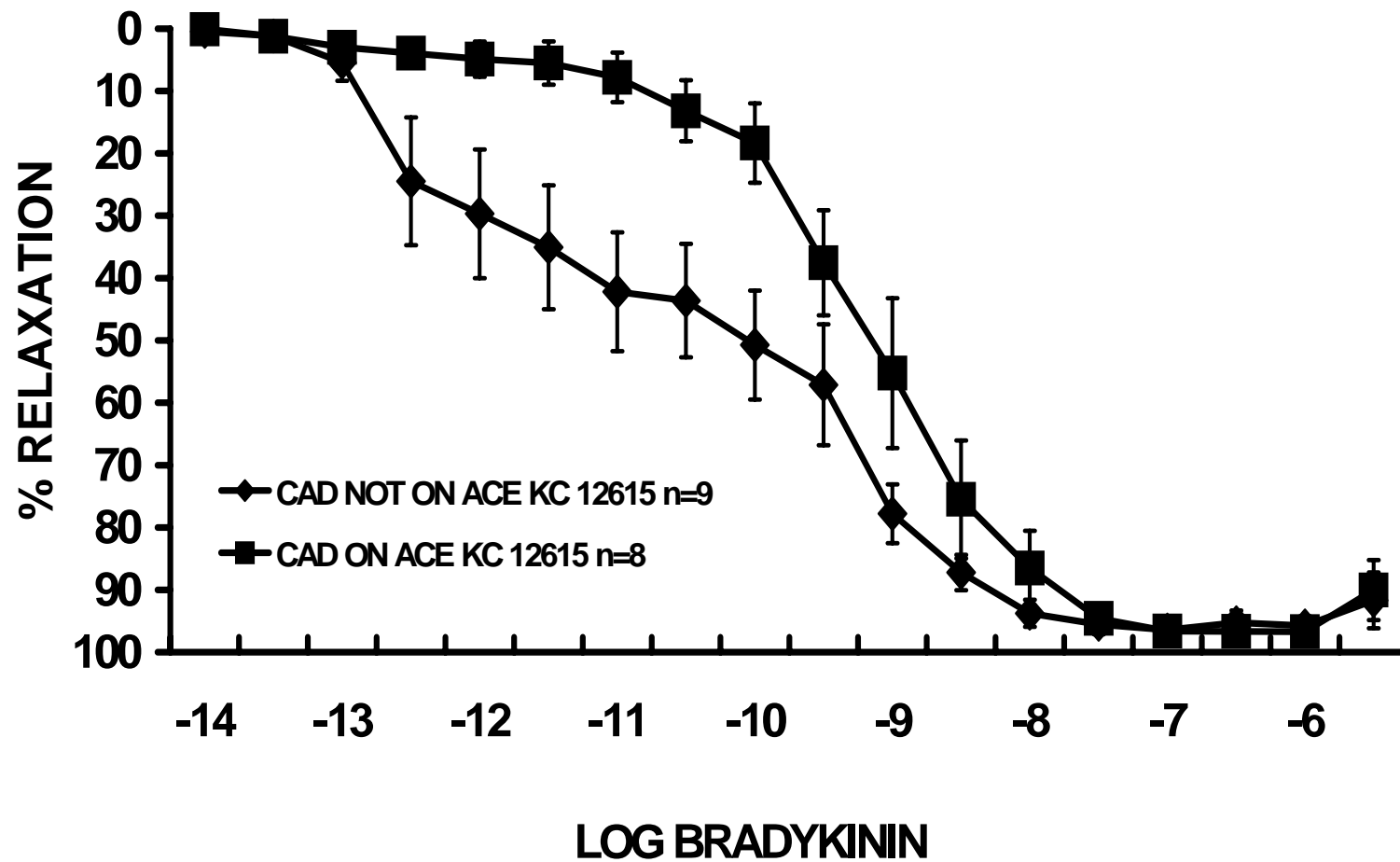
Cumulative concentration response curves (vasodilatation) for bradykinin (BK). Vasodilatation is expressed as percent relaxation relative to the baseline norepinephrine precontracted state. Bradykinin has a powerful vasodilator effect. This is significantly augmented by phosphoramidon, KC12615 and thiorphan (all  $p < 0.01$ ). [Includes patients taking and not taking ACE inhibitors]





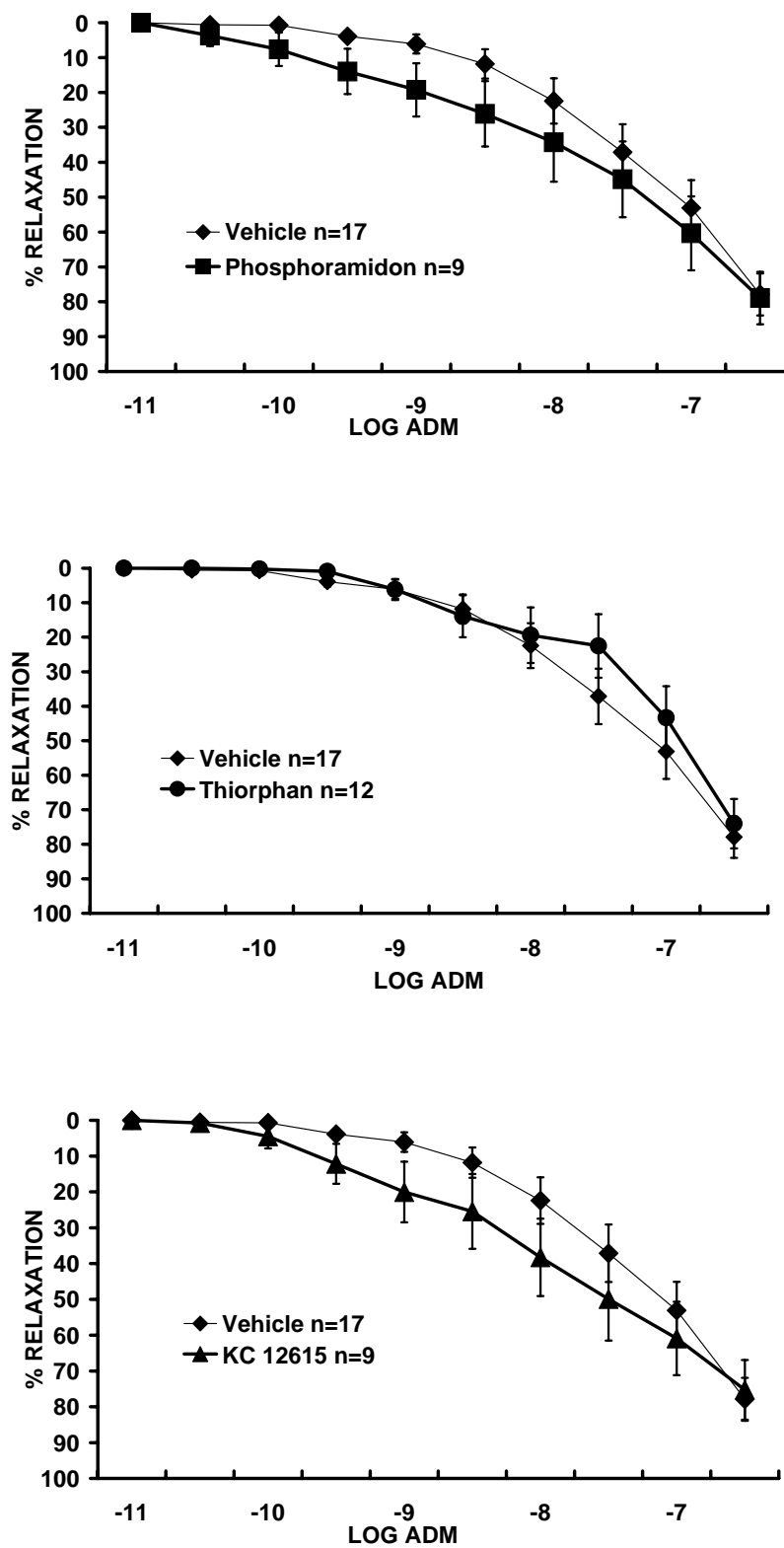
**Figure 5.4**

Cumulative concentration response curves (vasodilatation) for bradykinin. Small resistance arteries taken from patients pre-treated or not pre-treated with angiotensin converting enzyme inhibitors (ACE I). KC 12615 only enhanced the vasodilator response to bradykinin in patients not pre-treated with an ACE inhibitor ( $p < 0.05$ ).



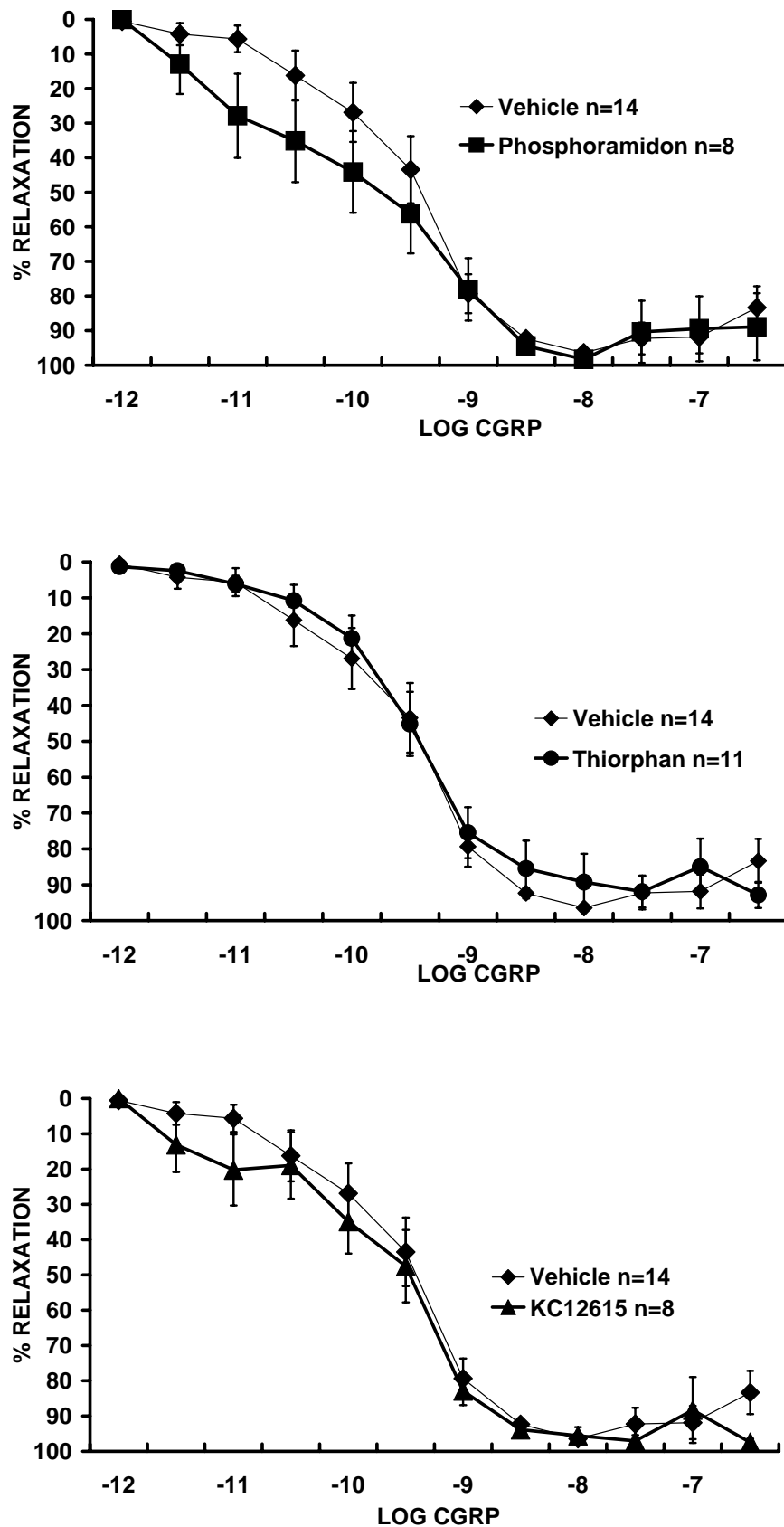
**Figure 5.5**

Cumulative concentration response curves (vasodilatation) for adrenomedullin (ADM). There is augmentation of the vasodilator effect of adrenomedullin by both phosphoramidon and KC12615 (both  $p < 0.05$ ), but not thiorphan.



**Figure 5.6**

Cumulative concentration response curves (vasodilatation) for calcitonin gene related peptide (CGRP). The relaxation response to calcitonin gene related peptide was significantly augmented by phosphoramidon only ( $p < 0.05$ ).



## Discussion

I have shown that KC 12615, the active metabolite of SLV306, has comparable activity to phosphoramidon, a well characterized inhibitor of NEP and ECE (Petrie, Hillier et al 2001, Jeng et al 1989, Ikegawa et al 1990). Phosphoramidon, however, is neither orally active nor available for human use. Though NEP inhibitors have been studied in humans, there are no reports of the use of orally active ECE inhibitors or dual NEP/ECE inhibitors. By contrast, SLV306 is orally active and has recently been given to healthy volunteers and patients. Other dual NEP/ECE inhibitors have been described, as has a triple enzyme (ACE/ECE/NEP) but none have been administered to humans (Trapani et al 1995, 2000, Ksander et al 1998).

I have found that  $10^{-4}$  M KC 12615 significantly attenuated the contractile response to big-ET-1, in keeping with inhibition of ECE mediated generation of ET-1.

In addition, phosphoramidon and KC 12615, but not thiorphan, enhanced the vasorelaxant action of ANP, though this action was less marked with KC 12615 than with phosphoramidon. Enhancement, of the action of ANP is consistent with inhibition of NEP and in keeping with the ability of SLV306 to augment blood natriuretic peptide concentrations *in vivo*.

What is of particular interest in my study, however, is the effect of KC 12615 on other vasoactive peptides. Prior studies with NEP inhibitors, in a variety of experimental models, have suggested that NEP is also capable of catabolising substances other than natriuretic peptides (Lewis et al 1997, Kokkonen et al 1999, Katayama et al 1991). For that reason I also studied the effect of KC 12615, phosphoramidon and thiorphan on the action of several other vasodilators. All three drugs markedly enhanced the vasorelaxant action of bradykinin. Interestingly, this action was only apparent in arteries taken from patients who had not been pre-treated with an ACE inhibitor. Clearly, ACE also known as kininase II, breaks down bradykinin and ACE inhibition enhances the action of bradykinin (Linz et al 1995). It seems that NEP inhibition does not further augment the effect of bradykinin when ACE has already been inhibited i.e. therapeutic ACE inhibition alone seems to maximally augment the action of bradykinin. This finding has potential clinical implications as it is

likely that any dual NEP/ECE inhibitor will be used mainly in addition to an ACE inhibitor. This may also allay concerns about angioedema (Messerli and Nussberger 2000). Of course, I have only studied small resistance arteries and the ratio of NEP and ACE differs in other tissues e.g. the myocardium (Blais et al 2000). Consequently, the effect of combined therapy with an ACE inhibitor and a dual NEP/ECE inhibitor could differ between tissues.

Interestingly, I also found that phosphoramidon and KC 12615 (but not thiorphan) augmented the action of ADM, a recently described and powerful endogenous vasodilator which may protect against cardiovascular damage (Shimosawa et al 2002). NEP inhibition has been reported to increase plasma concentrations of ADM in sheep and enhance the renal actions ADM in dogs (Ily et al 1998, Rademaker et al 2002). I have now shown this action in human blood vessels. Phosphoramidon (but not thiorphan or KC 12615) also enhanced the action of CGRP, a peptide sharing structural homology with ADM.

These actions of phosphoramidon and KC12615, in addition to the attenuation of big ET-1 mediated vasoconstriction, almost certainly reflect inhibition of NEP. Thiorphan, however, did not share these actions, other than in the augmentation of the vasodilator effect of bradykinin. This may be because thiorphan is a relatively weak NEP inhibitor.

This unexpectedly wide array of actions of effective dual NEP and ECE inhibitors makes such compounds, potentially, very attractive therapeutic agents. Diminished ET-1 production, coupled with an augmented effect of natriuretic peptides, bradykinin and ADM, is of theoretical benefit in hypertension, heart failure, atherosclerosis (including saphenous vein grafts (Porter et al 2001) and, perhaps, renal failure and diabetes (Tikkanen et al 2002). However, such benefits have to be demonstrated in properly designed clinical trials.

In summary, my findings show that dual NEP and ECE inhibitors can modulate the action of vasoactive mediators other than ET-1 and natriuretic peptides. These further actions may indicate that this new class of pharmacological agent has anti-hypertensive, cardiovascular and renal benefits in addition to those anticipated.

## **6 A study to establish the pressor response to big endothelin-1 infusion in healthy volunteers.**

### **Background**

ET-1, a neurohumoral peptide, is a powerful vasoconstrictor, mitogenic and anti-natriuretic peptide which is produced in increased quantities in CHF. ET-1 is also believed to contribute to the development and progression of atherosclerosis and may have a role in the development of hypertension. ET-1 is generated from a precursor peptide, big ET-1, by a specific ECE. Studies in vivo have demonstrated a potent vasoconstrictor effect of both ET-1 and its precursor big ET-1, with both peptides causing significant increase in mean arterial pressure (MAP).

Currently there is much interest in the development of anti-endothelin drugs for the treatment of heart failure in particular and cardiovascular disease in general. One such group of drugs are ECE Inhibitors. To be able to evaluate the efficacy and dose response relationships for these new compounds it is necessary to demonstrate their ability to blunt the BP rise induced by big ET-1. In turn this requires an understanding of the relationship between big ET-1 dose and arterial pressure response.

The purpose of this study is to find a dose of big ET-1 that gives a measurable but modest increase in arterial pressure in healthy volunteers. This would then permit evaluation of the efficacy and dose response relationship of SLV 306, a novel neurohumoral peptide with dual NEP and ECE inhibitory action.

In previous studies a rising dose regime of 0.2, 1 and 8 pmol/kg/min, for 20 minutes each has been associated with a maximum increase in MAP of 33mmHg (Ahlborg et al 1994, 1996, Pernow et al 1996). In my pilot studies however this regime did not lead to a measurable increase in blood pressure (monitored for 2 hours post- infusion). I aimed in this study to find a dose of Big ET-1 which consistently causes a blood pressure rise of 20 – 30 mmHg in the majority of volunteers.

## **Methods**

The design of this study was based on similar studies using angiotensin I, routinely carried out to determine pressor-dose response curves. These curves were then used to test the effect of ACE inhibitors. The Big ET-1 effect on blood pressure, however, is slower than that of angiotensin I and is sustained for about 90 minutes, preventing the use of a large range of doses. I therefore monitored the blood pressure at rest and following infusion of only three doses of Big ET-1 in 6 healthy male volunteers

### Healthy volunteers

Six healthy male volunteers were enrolled following a screening visit to ensure eligibility. They were recruited from a volunteer database held in the research unit. Six has been an adequate number, to give significant results, in previous studies using Big ET-1.

### Big endothelin infusions

During the study session subjects were given three systemic, intravenous infusions of big ET-1.

The first infusion was at a rate of 8 pmol/kg/min as given in previous studies, the second was at a rate of 10 pmol/kg/min. and the third was at a rate of 12 pmol/kg/min. Each infusion lasted for 20 minutes in total.

### **Blood pressure measurements**

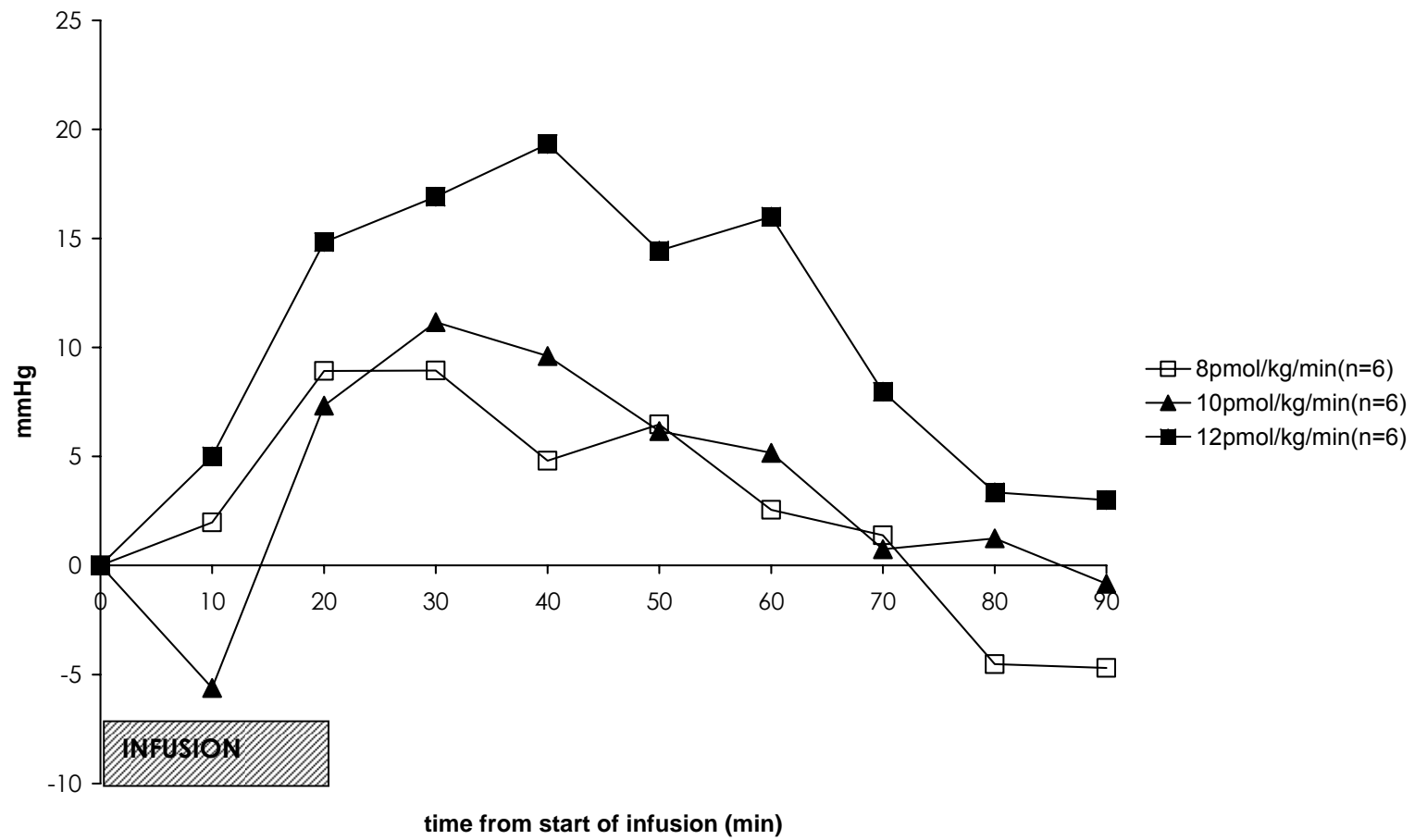
Blood pressure measurements were made at ten minute intervals from 30 minutes before until 2 hours after each infusion or until baseline values were obtained.

## **Results**

I demonstrated that the highest Big ET dose of 12 pmol/kg/min gave a clear pressor response, whilst the 8 and 10 pmol/kg/min-doses showed a smaller effect (See figure 6.1).

**Figure 6.1**

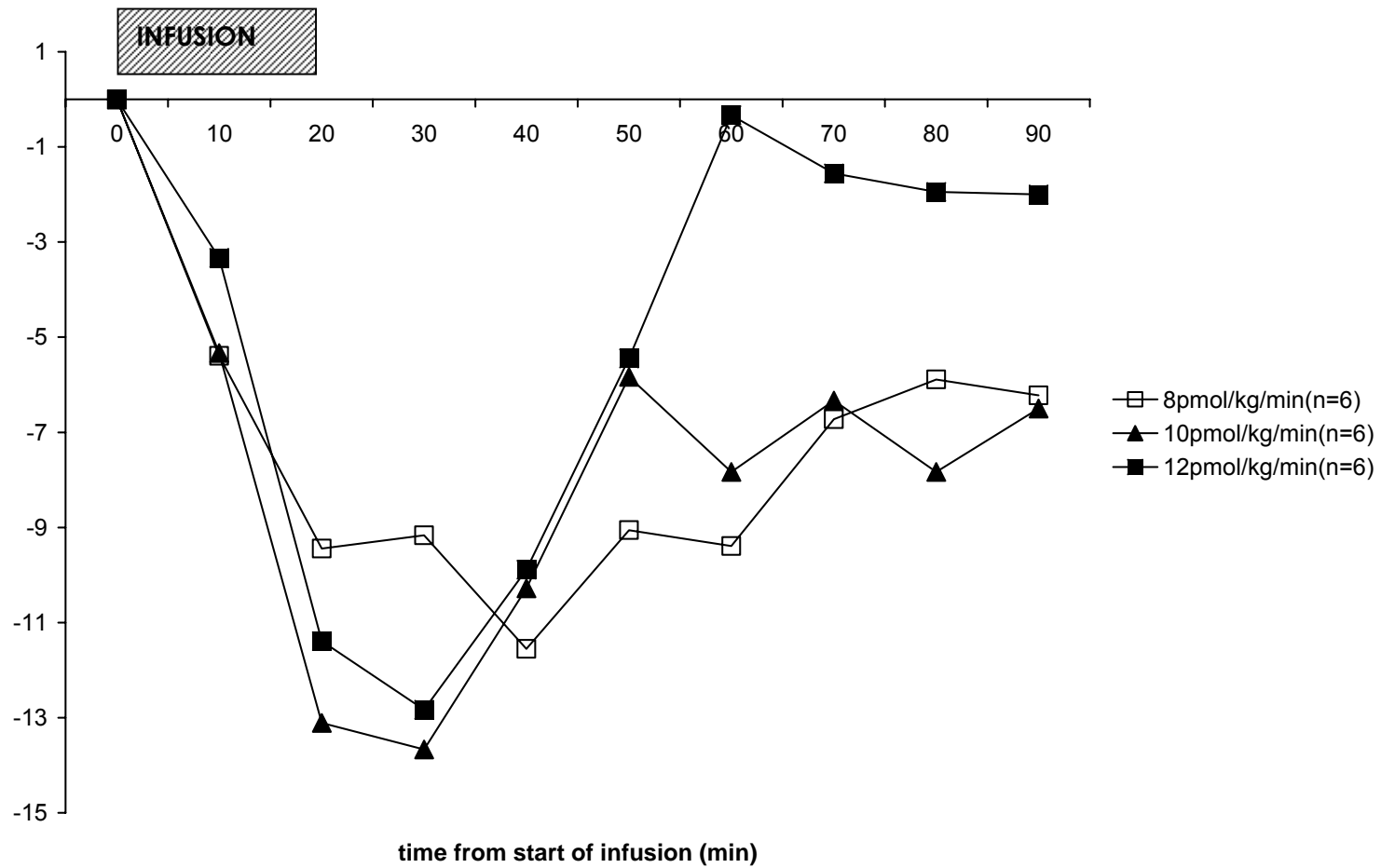
**The effect of three doses of big endothelin-1 on the mean arterial pressure of healthy volunteers (n=6)**





**Figure 6.2**

**The effect of three doses of big endothelin-1 on the heart rate of healthy volunteers (n=6)**



## **Discussion**

Overall, the results were not as impressive as described in the literature (Ahlborg et al 1994, 1996, Pernow et al 1996). There was little difference in response between the two lower doses.

Despite rigorous assessment of the methods used I was unable to explain this. I used the same infusion methods, the same controlled conditions and the same non invasive method of assessing blood pressure.

The pressor response to the higher dose was accompanied by the expected reflex bradycardia (see figure 6.2). With respect to both blood pressure and heart rate, the response to the highest big ET dose was large enough and most importantly reproducible enough to reliably show an influence on it by pharmacological intervention.

This dosing study allowed me to choose doses of big ET for my SLV 306 dose response study which I knew were associated with clear reproducible pressor and heart rate response in my hands.

## **7 Dose response study with SLV306 in healthy human volunteers: effects on haemodynamic responses to big ET-1 infusion and hormonal actions**

### **Background**

SLV 306 is a molecule that has been shown to inhibit both ECE and NEP in animal tissues. ECE converts the inactive propeptide big ET-1 to ET-1. NEP breaks down ANP, BNP and CNP. SLV 306, therefore reduces ET-1 production and increases natriuretic peptide concentrations. SLV 306 is the first orally active dual ECE/NEP inhibitor in development as a therapeutic agent (see section 1.3.4).

NEP inhibitors were ineffective anti-hypertensives, probably because NEP catabolises ET-1. Dual NEP and ECE inhibition may be more useful therapeutically.

The aim of this study was to establish the dose response (concentration response) relationship for the ECE and NEP inhibitor activity of SLV 306 in healthy volunteers. This is presently unknown but clearly key to choosing the appropriate dose range of SLV 306 for therapeutic studies in patients. One way in which I will assess the ECE inhibitory action of SLV 306 is to evaluate the effect of three plasma concentrations of the main metabolite of SLV 306 (KC 12615) on the pressor response to big ET-1. The approach used is analogous to that used to study dose response relationships for ACE inhibitors (Biollaz et al 1981, Burnier et al 1981, Wellstein et al 1987). I will also study the effect of the three doses of SLV 306 on neurohormones.

The target parameters are the change from baseline in the MAP and the plasma levels of the neurohormones big ET-1, ET-1 and ANP before, during and after the big ET-1 infusion.

### **Methods**

There were two parts to this study.

Due to a high inter-subject variability in the plasma concentrations of SLV306 and KC12615, a concentration controlled design was used. 29 Male volunteers attended for the first stage of the study which involved taking two different doses of SLV306

(400mg (n=29), and 600mg (n=6) or 800mg (n=23)) at least seven days apart. Blood samples were collected after dosing for measurement of plasma concentrations of KC12615. Pharmacokinetic modelling was used to calculate the individual doses of SLV306 needed to achieve average plasma concentrations of KC12615 of approximately 75ng/ml, 300ng/ml and 1200ng/ml over the first 6 hours. These values correspond roughly with the expected mean KC 12615 concentrations following single doses of 100 mg, 400 mg and 800 mg respectively of the parent compound SLV 306.

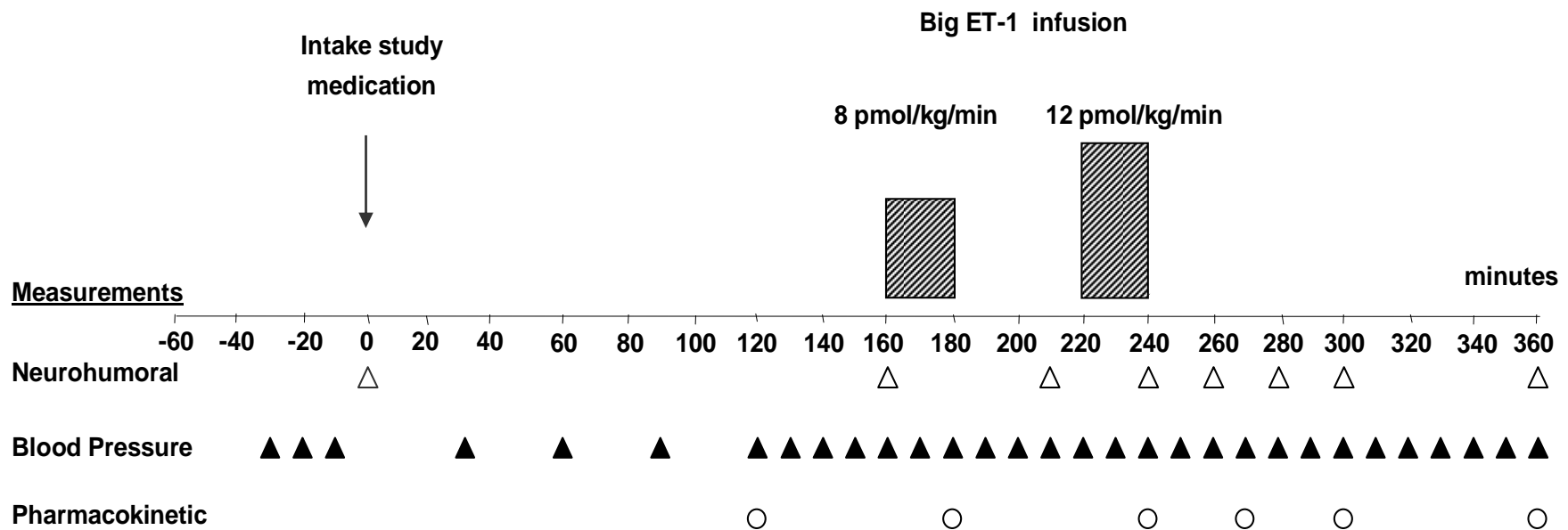
The second part of the study was a double blind, placebo controlled, four period cross-over study in 15 healthy male volunteers (mean age 22, range 18-38 years). It involved four further visits at least seven days apart. For these, volunteers attended the clinical laboratory at 08.00h, having fasted from midnight. An intravenous cannula was placed in each forearm. After one hour of supine rest, baseline blood pressure and heart rate recordings were made and blood samples taken (see below). Each subject then received either placebo or a single oral dose of SLV306. A double-blind ascending dose protocol with random insertion of placebo was used to assign treatment. 160 minutes after dosing, a 20 minute infusion of 8 pmol/kg/min of big ET-1 was administered (figure 7.1). After a further 40 minutes (220-240 minutes post-dosing) a second 20 minute infusion of 12 pmol/kg/min of big ET-1 was given.

#### Healthy volunteers

29 healthy male volunteers were enrolled for the first part of the study following a screening visit to ensure eligibility. 15 of these proceeded to the second part of the study. Subjects were excluded from the principal study if they had no measurable plasma levels or they needed more than 1600mg SLV 306 to achieve a plasma level in the required range. 15 subjects was thought to be an appropriate number since a similar number gave significant results, in studies of a similar type involving ACE inhibitors (Biollaz et al 1981, Burnier et al 1981, Wellstein et al 1987).

**Figure 7.1**

Study protocol. Subjects rested supine for 60 minutes before intake of study medication. The first big endothelin-1 infusion was commenced 160 minutes after dosing and the second after 220 minutes.  $\Delta$  = neurohumoral  $\blacktriangle$  = blood pressure and  $\bigcirc$  = pharmacokinetic measurements.



## SLV 306

In the pilot part of the study all 29 subjects were given 400mg on day 1, 6 of the subjects were then given 600mg on day 8. Once this dose was seen to be well tolerated, the remaining 23 subjects were given 800mg. This was for safety as the highest dose of SLV 306 previously given to humans was 400mg.

In the principal part of the study the doses given were adapted individually. The doses given were those found to achieve target plasma concentrations of KC 12615 of 75ng/ml, 300ng/ml, and 600ng/ml (comparable to 100, 400 and 800mg of SLV 306 respectively) at the infusion time i.e. 3 hours post dose.

Subjects were excluded if they had no detectable plasma levels or if they need more than 1600mg SLV 306 to achieve a plasma level in the required range.

15 subjects who received 800 mg in the pilot part of the study (unless excluded) proceeded to the principal study, allowing up to eight subjects to remain in reserve, available to replace subjects that no longer complied with the study requirements.

The subjects in the principal study were given one of the three doses of SLV 306 or placebo, on each study day, blinded in a double blind fashion.

## Big Endothelin-1 infusions

In each of the principal study sessions subjects were given a systemic, intravenous infusion of big ET-1 160 minutes after dosing with placebo or SLV306. The infusion was commenced at a rate of 8.0pmol/kg/min. After 40 minutes (220-240 minutes post dosing) the infusion rate increased to 12pmol/kg/min and this rate of infusion continued for 20 minutes before being stopped. The doses of big ET-1 were chosen from the pilot study (chapter 6), as ones likely to lead to a rise in mean arterial pressure of approximately 20 mmHg. The timing of the infusions was chosen to coincide with peak plasma concentrations of KC12615 as demonstrated in the pharmacokinetic first stage of the study.

### Measurements of arterial pressure and heart rate

Heart rate and arterial pressure measurements were made at ten minute intervals initially, then every 30 minutes until the start of the big ET-1 infusion. From the start of the infusion they were again taken every ten minutes until two hours after completion of the infusion (figure 7.1).

### Blood samples for neurohumoral measurements

11ml of venous blood was collected from the contralateral forearm into chilled tubes at baseline and 160, 210, 240, 260, 280, 300 and 360 minutes after administration of study drug (figure 7.1).

Plasma big ET-1 and ET-1 levels were measured to assess the effect of SLV 306 and its metabolite on the conversion of big ET-1 to ET-1. Plasma ANP concentrations serve as a measure of the degree and time course of NEP inhibition.

Samples were handled, stored and analysed as described in chapter 2.

### Statistical methods

The treatment groups were compared using a two-way ANOVA with factors for treatment group, study period and subjects. In addition, least square mean changes were calculated for each treatment group and the three SLV 306 minus placebo differences were estimated. Each of the three comparisons was performed at the 5% significance level, without adjustment for multiple testing.

## **Results**

Of the 15 volunteers taking part in the big ET-1 infusion studies, 1 withdrew for personal reasons and 1 was withdrawn because of an excessive rise in blood pressure (30 mmHg) and fall in heart rate (-15 beats/min). No subjects experienced any significant adverse event.

Plasma concentrations of KC12615: Maximum plasma concentrations for KC 12615 were achieved at about 3-4 hours following oral dosing of SLV 306. The low, medium and high doses of SLV 306 resulted in clearly distinguishable plasma concentrations of

KC 12615. The respective mean steady state concentrations were 30, 252 and 1674 ng/ml.

Arterial pressure: The rise in systolic and diastolic arterial pressure following big ET-1 infusion is shown in figure 7.2. SLV 306 caused a dose dependent attenuation of the hypertensive response to big ET-1. The mean peak (SE) increases in systolic, diastolic and mean arterial pressure were 19.2(2.1), 16.1 (1.5) and 15.9(1.6) mmHg after placebo pre-treatment. The increases after 75ng/ml were, 18.3(3.0), 14.0 (2.1) and 15.0 (2.3) mmHg, respectively. The respective increases after 300ng/ml were 15.5(2.5), 13.3 (1.7) and 13.5(1.9) mmHg. Those after 1200ng/ml were 10.4(2.8), 10.5(2.0) and 9.0 (2.1) mmHg, respectively. The differences between placebo and 1200 ng/ml were all significant for systolic, diastolic, and mean arterial pressure.

Heart rate: SLV 306 caused an inhibition of the reflex bradycardia induced by big ET-1 infusion (figure 7.2). During big ET-1 infusion, the mean peak decreases in heart rate were 12.1 (1.1), 11.2 (1.5), 10.2 (1.2) and 7.9 (1.4) beats per minute after pre-treatment with placebo, 75, 300 and 1200ng/ml, respectively.

Atrial natriuretic peptide (ANP): SLV 306 led to a dose dependent increase in plasma ANP concentrations (figure 7.3), consistent with systemic NEP inhibition. The mean peak (SE) increases in plasma ANP after placebo, 75, 300 and 1200 ng/ml were 5.4 (3.0), 11.0 (4.2), 24.0 (3.5) and 20.6 (4.0) pg/ml, respectively. The between-group differences for 300 ng/ml and 1200 ng/ml compared to placebo were statistically significant: 18.6 (4.7) [p=0.0004] and 15.3 (4.8) [p=0.003], respectively.

Big ET-1 and ET-1: SLV 306 caused a dose dependent increase in plasma big ET-1 concentrations during and after the big ET-1 infusions (figure 7.3). The mean (SE) increases in plasma big-ET in the first hour following the completion of the big-ET infusions after treatment with placebo or 75, 300, and 1200 ng/ml were 96.3 (18.3), 128.6 (25.8), 184.4 (21.6) and 216.1 (24.3) pmol/l. The between group differences versus placebo were statistically significant for the 300 ng/ml and 1200 ng/ml doses: 88.1 (28.6) [p=0.004] and 119.9 (29.6) fmol/l [p=0.0003], respectively.

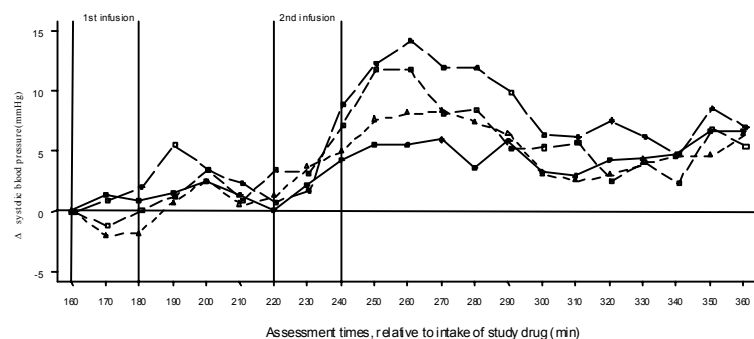
There was no evidence of any between group differences for plasma ET-1 levels during the same period.



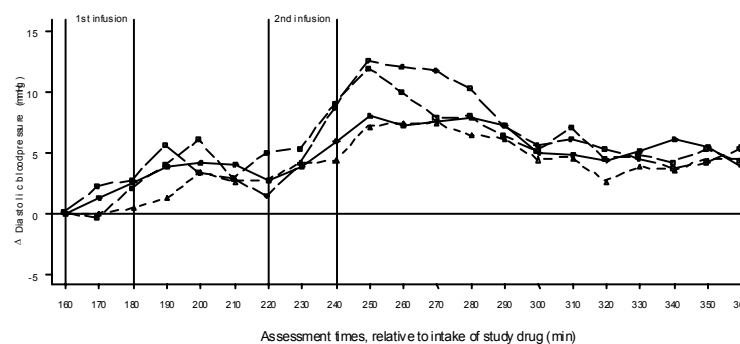
**Figure 7.2**

Change in (a) systolic, (b) diastolic and (c) mean arterial pressures, from baseline, during and after big endothelin-1 (big ET-1) infusion on each of the four study days (i.e. after pre-treatment with placebo or SLV 306) to give KC 12615 plasma concentrations of approximately 75, 300 and 1200mg/ml. "Baseline" was the average of the last 3 arterial pressure measurements made prior to commencement of the first big ET-1 infusion. Change in heart rate (d) is shown in the same way

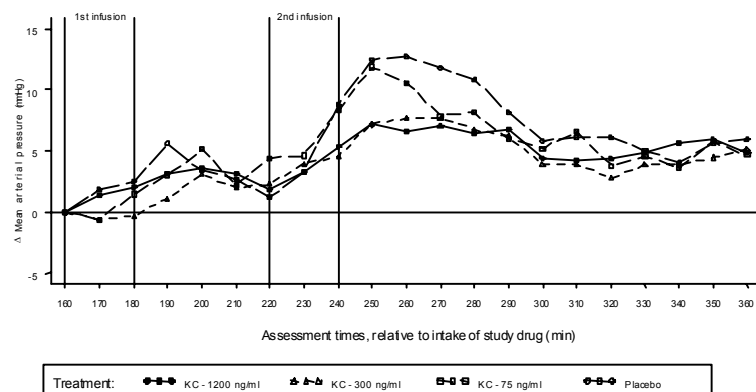
**a) Change in systolic arterial pressure\***



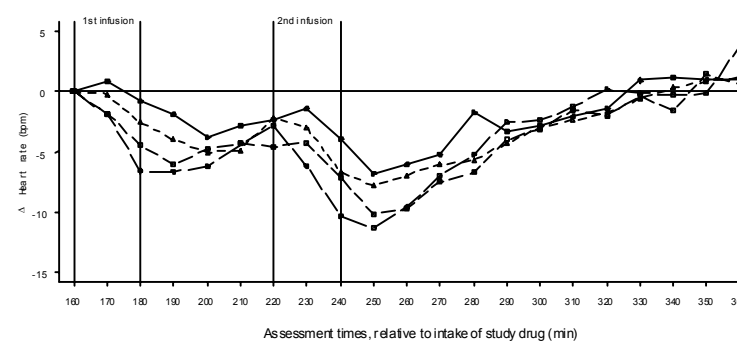
**b) Change in diastolic arterial pressure\***



**c) Change in mean arterial pressure\***



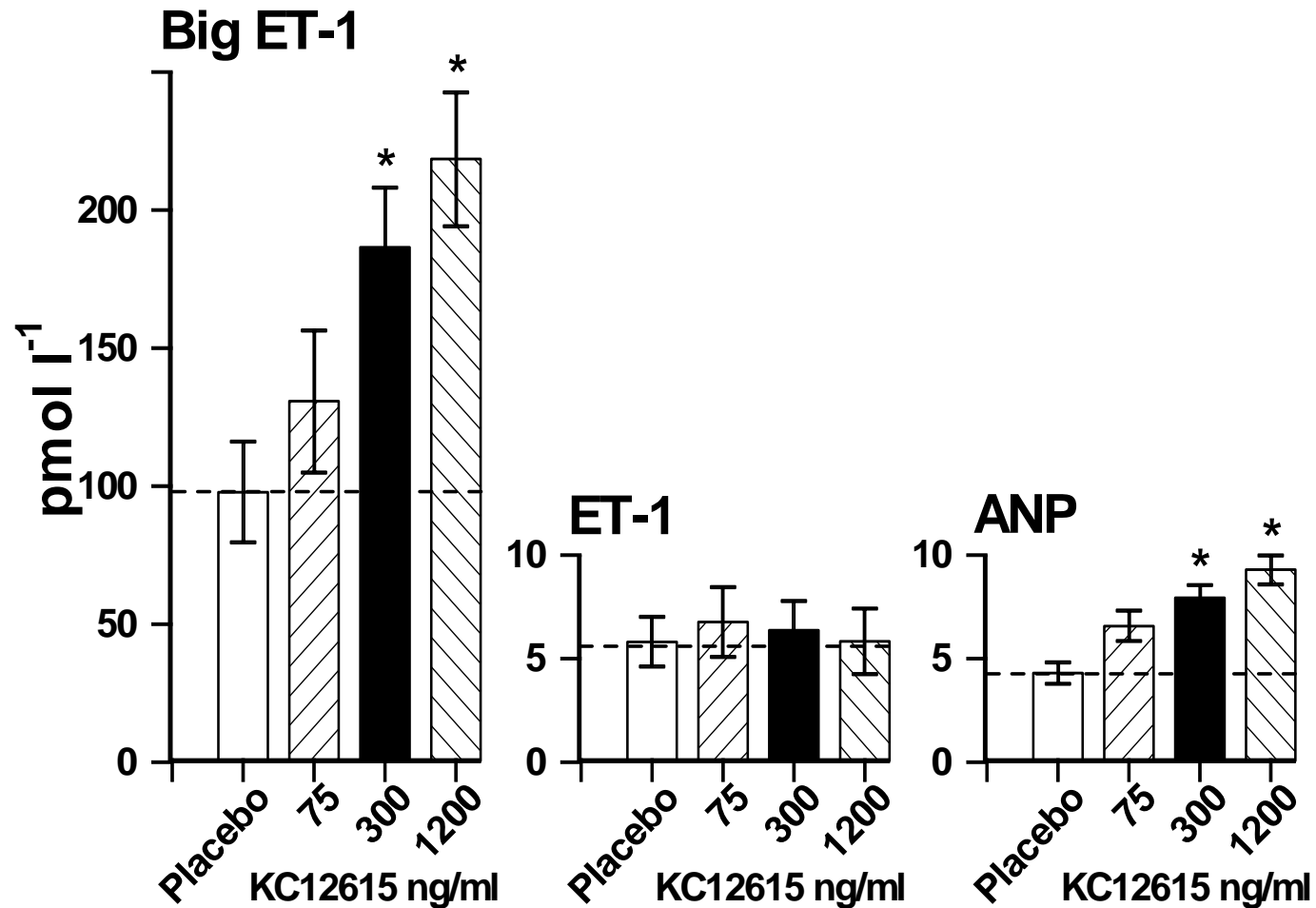
**d) Change in heart rate\***



\* Corrected for baseline before the start of the 1st infusion (average of 140,150,160 minute measurements)

**Figure 7.3**

Hormonal changes following oral dosing with placebo or SLV 306 and subsequent infusion of big endothelin-1. Changes in plasma Big ET-1, ET-1, ANP peptide levels in response to increasing doses of KC12615 (the active metabolite of SLV306). Each value represents the mean increase in plasma levels measured in 13 volunteers in the first hour following completion of the big ET-1 infusion in either placebo (representing mean basal conversion indicated by horizontal line) and each of the increasing doses. There was a significant dose dependent rise in Big ET-1, (\*P<0.005) consistent with inhibition of ECE, and a rise in ANP consistent with inhibition of NEP activity. ET-1 levels were unaltered.



The ratio of big ET-1 to ET-1 dose dependently increased, consistent with systemic ECE inhibition preventing metabolism of the enzyme substrate (big ET-1) to its active metabolite (ET-1, figure 7.3). The mean (SE) ratio of plasma big-ET-1/ET-1

concentration increased in a concentration dependent manner following the big ET-1 infusion: 21.6 (3.0), 20.1 (4.2), 34.1 (3.5), and 41.5 (3.9) after placebo, 75, 300 and 1200 ng/ml, respectively. The between group differences versus placebo were statistically significant for the 300 ng/ml and 1200 ng/ml doses: 12.5(4.6) [p=0.011] and 20.0 (4.8) [p=0.0002], respectively.

## Discussion

I have studied the first in a new class of orally active dual metalloprotease inhibitors. SLV306 and its active metabolite have *in vivo* pharmacologic actions consistent with inhibition of both NEP and ECE.

I employed a similar experimental approach to that used to demonstrate the pharmacologic actions of ACE inhibitors (and dual NEP and ACE inhibitors) to confirm any ECE inhibiting action of SLV306 *in vivo* (Rousso et al 1998, Jardine et al 1990). I have shown that SLV306 dose dependently blocks the pressor response to big ET-1, in keeping with functional ECE inhibition *in vivo*. NEP inhibition alone might have been expected to augment the hypertensive effect of big ET-1 infusion (Ando et al 1995, McDowell et al 1997). I also found that plasma concentrations of big ET-1 increased dose dependently on the SLV306 days when compared with the placebo day. Indicating that SLV 306 was inhibiting an increasing proportion of endogenous ECE activity. Interestingly, despite inhibition of NEP there was no significant change in plasma ET-1 concentration during big ET-1 infusion. NEP is thought to metabolise ET-1 to biologically inactive fragments. Moreover, the big ET-1/ET-1 ratio significantly increased consistent with reduced conversion of big ET-1 to ET-1. Kuc et al have since carried out further analysis from our study which supports this interpretation. ECE is responsible for the conversion of big ET-1 to the active peptide ET-1 and the C terminal fragment (CTF). They measured CTF in blood samples taken from our study subjects. They demonstrated a significantly smaller rise in CTF in the presence of SLV 306 than in the presence of placebo (Kuc et al 2005).

I was also able to confirm that SLV306 is a NEP inhibitor *in vivo*. SLV306 caused a dose dependent increase in plasma ANP concentration almost identical to that obtained with candoxatril in healthy volunteers (i.e. an approximate doubling of plasma ANP concentrations with the highest dose of inhibitor) (Northridge et al 1989, Jardine et al 1990).

In conclusion, I believe that an agent that is both a NEP inhibitor, augmenting circulating natriuretic peptide production *and* an ECE inhibitor, reducing ET-1 production, is an attractive therapeutic option in a range of cardiovascular diseases. Such an agent overcomes the pharmacologic limitations of a sole NEP inhibitor, especially if used in combination with an ACE inhibitor or angiotensin II receptor antagonist.

## **8 A double blind, randomised, three-way crossover study in healthy human volunteers to compare the effects of two doses of GW660511X with ramipril on NEP and ACE activity**

### **Background**

GW660511X ('511) is an inhibitor of both ACE and NEP. The conversion of angiotensin I to angiotensin II is mediated by ACE. NEP breaks down ANP, BNP and CNP. '511 therefore reduces angiotensin II production and increases natriuretic peptide concentrations.

The pharmacodynamic profile of neither the ACE inhibitor activity nor the NEP inhibitor activity of '511 has been fully described in humans. Initial studies have characterized the ACE inhibition by assessing plasma ACE activity and have partially characterized markers of NEP inhibition. This study is intended to compare the ACE inhibition of '511 with a clinically relevant comparator, ramipril. The challenge for the development of vasopeptidase inhibitors, as useful therapy in hypertension or heart failure, has been the need for demonstration of a beneficial effect over and above ACE inhibitors.

The aim of this study was to compare ACE inhibition by plasma ACE activity and the in vivo haemodynamic effect of angiotensin I infusion. NEP inhibitor activity was further characterized by measuring plasma cGMP and plasma ANP.

The study compares two dose levels of '511, 100 and 200mg, with a clinically relevant dose of the ACE inhibitor ramipril, 10mg.

The design of this study is based on similar studies using angiotensin I which were carried out to determine the dose response relationships for ACE inhibitors (Biollaz et al 1981, Burnier et al 1981, Wellstein et al 1987).

## Methods

### Healthy volunteers

18 healthy male volunteers were enrolled following a screening visit to ensure eligibility. Based on this sample size it was anticipated that at least 15 subjects would complete the clinical phase of the study (i.e. complete all treatment periods). 15 subjects was thought to be an appropriate number since a similar number gave significant results, in studies of a similar type involving ACE inhibitors (Biollaz et al 1981, Burnier et al 1981, Wellstein et al 1987).

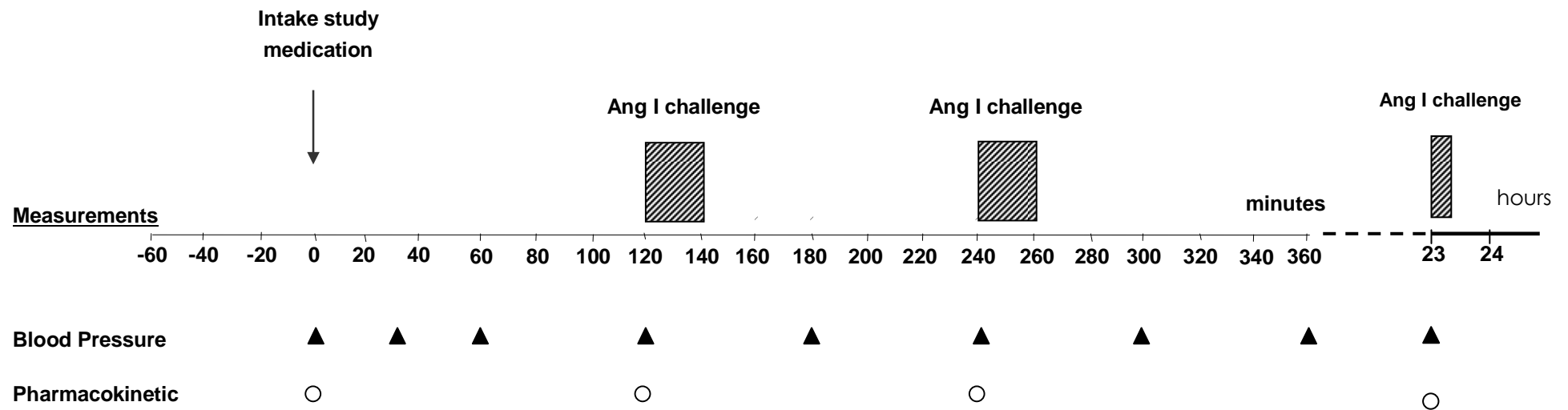
Following screening and then a preliminary visit which included an angiotensin I infusion challenge to check for hypertensive response (detailed below), each subject was given seven daily doses of either 10 mg ramipril or 100 mg '511 or 200 mg '511 during each day of three seven day study periods. The volunteers were asked to attend each morning for observed dosing. All study periods were conducted identically.

On day 4 of each seven day period, the subjects should have reached steady state (based on the half life of '511 (12 hours) and ramipril (11 hours)). Day 4 was therefore chosen for the angiotensin I challenge. Volunteers were asked to attend the research unit for 24 hours on this day. During this visit angiotensin I was administered at 2, 4 and 23 hours post dosing. These times were chosen to represent the predicted peak (2 hour), trough (23hours) and a convenient intermediate time point (figure 8.1). The dose of angiotensin I ( $\mu\text{g}/\text{min}$ ) which increased systolic or diastolic blood pressure to a predefined level [infusion stopped if systolic BP increased to 180 mmHg or above (or by 40 mmHg or more) or diastolic BP increased to 110 mmHg or above (or by 25 mmHg or more)] in the presence of '511 or ramipril was determined. These angiotensin I dose level values were compared to the angiotensin I dose level value which increased systolic or diastolic blood pressure to the predefined safety levels prior to drug treatment (baseline). Mean (95% confidence interval) angiotensin I dose-blood pressure response (DR-1) for each drug at each post-dose time point were determined and plotted against time. Individual DR – 1 values were plotted against individual '511 concentration values.

**Figure 8.1**

Study day 4 protocol. Subjects rested supine for 60 minutes before intake of study medication. The first angiotensin I challenge was commenced 120 minutes after dosing, the second after 240 minutes and the third after 23 hours.

▲ = blood pressure and ○ = pharmacokinetic measurements and Ang I = angiotensin I



**Table 8.1**

Study day 7. Time and Events Schedule

In bed for first 8 hours including giving urine samples Then for 1 hour before each blood sample	In bed until after 1330 blood sample <u>Lunch</u> after 230pm <u>Evening meal</u> after 530pm									In bed 1630	In bed 2030	In bed 1230	In bed 0830
<b>Real Time</b>	<b>0830</b>	<b>0925</b>	<b>0930</b>	<b>1000</b>	<b>1030</b>	<b>1130</b>	<b>1230</b>	<b>1330</b>	<b>1430</b>	<b>1730</b>	<b>2130</b>	<b>0130</b>	<b>0930</b>
	-1h	Pre- dose	0	0.5h	1h	2h	3h	4h	5h	8h	12h	16h	24h
Resting Semi-Recumbent	X	X	X	X	X	X	X	X	X	X			
Study Medication Administration			X										
Drink water	0.5L				0.2L	0.2L	0.2L	0.2L	0.2L	0.2L			
Pharmacodynamic Blood Sample		X		X	X	X	X	X		X	X	X	X
Pharmacokinetic Blood Sample		X		X	X	X	X	X		X	X	X	X
AE collection	X	X	X	X	X	X	X	X	X	X	X	X	X



A two day period was left between the angiotensin I challenge and the next assessment to allow any effects of angiotensin I on hormones associated with renal handling to resolve.

On day 7 volunteers were again asked to attend the research unit for 24 hours, a 24-hour pharmacokinetic profile and a 24-hour ACE & NEP inhibition profile were obtained (figure 8.2). There was then a washout period of at least one week between study periods.

5 to 10 days following the last study session subjects returned for a follow-up visit.

### Angiotensin I infusions

Angiotensin I was given 2 hours, 4 hours and 23 hours after study medication was administered (figure 8.1). It was given by ascending dose infusion as described in chapter 2. During screening each volunteer received an angiotensin infusion according to this protocol and a rise in systolic blood pressure of at least 10mmHg was required for eligibility.

### Measurements of arterial pressure and heart rate

Heart rate and arterial pressure were measured in the last thirty seconds of each dose of angiotensin I and for safety reasons within the first minute of each increase in infusion rate.

### Blood samples for pharmacokinetic assessments

Blood samples (4 ml) for measurement of plasma '511 concentration were collected on four occasions on day 4 and on ten occasions on day 10.

### Blood samples for neurohumoral measurements

Blood samples (13 ml) for measurement of plasma ACE activity, plasma ANP and plasma cGMP were collected on ten occasions on day 7 (table 8.2). These neurohumoral analyses were taken as a measure of the degree and time course of ACE and NEP inhibition. Samples were handled, stored and analysed as described in chapter 2.

### Statistical methods

The primary comparisons of interest were between '511 100 mg and '511 200 mg and ramipril 10 mg, in terms of ACE activity. Least square means were calculated for each treatment group and the two '511 doses minus ramipril differences were estimated. Treatment ratios of all comparisons were calculated by taking the anti-log of the difference between the least squared means. Using pooled estimates of variance, 95% confidence intervals were

calculated for the difference then anti-logged. Comparisons were presented as a treatment ratio.

## Results

A total of 16 subjects were enrolled in the study.

### Plasma concentrations of GW660511X:

Maximum plasma concentrations of '511 were achieved at about 3 hours following oral dosing. The two doses of '511 (100mg and 200mg) resulted in approximately proportionate maximum and minimum plasma concentrations. The respective mean maximum concentrations were 10.9 and 25.1 µg/ml. The respective mean minimum concentrations were 1.1 and 2.54 µg/ml.

### Arterial pressure:

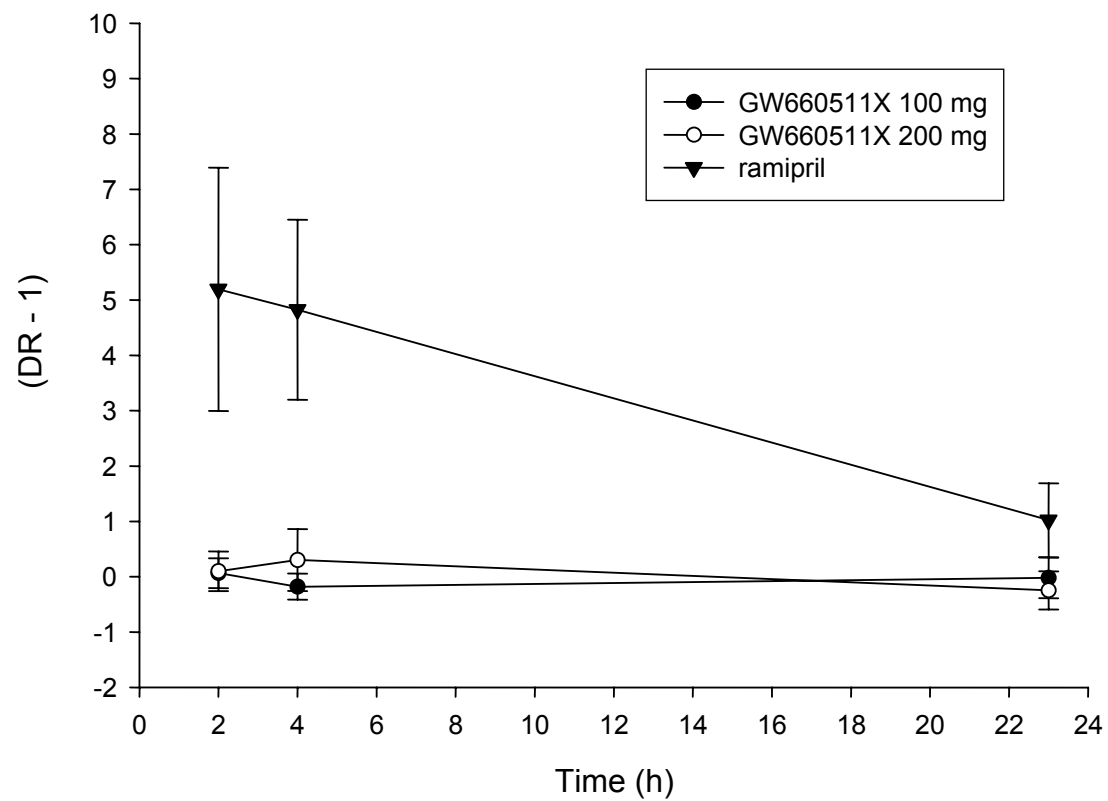
There was no notable change from baseline (0 hours) mean systolic or diastolic blood pressure at 2, 4 and 23 hours for the '511, 100 mg and 200 mg treatment groups (pre angiotensin I infusion). There was a decrease in mean systolic and diastolic blood pressure at 2 and 23 hours post-dose in the ramipril 10 mg treatment group (pre angiotensin I infusion).

Mean (95% confidence interval) angiotensin I DR-1 (dose response ratio -1) values at different times after administration of '511 and ramipril are shown in figure 8.3. Maximum effect for ramipril was observed 2 hours post-dose. This was followed by a time dependent reduction in the effect of ramipril with some inhibitory activity still apparent at 23 hours post-dose. For '511, only minimal inhibitory activity was observed at any given, post-dose time point.

### Serum ACE activity:

There was a decrease in baseline median ACE activity from Day 1 to Day 7 for all three treatment groups. The greatest change from baseline median ACE activity was following treatment with ramipril 10 mg; following '511 100 mg and '511 200 mg no significant change was detected.

Thus starting (baseline) median ACE activity was different for the three treatment groups on Day 7; '511 100 mg was 29.5 u/l, '511 200 mg was 20.0 u/l whereas that for ramipril 10 mg was 8.0 u/l.



**Figure 8.2** Study day 4: Mean (95% confidence interval) angiotensin I dose-blood pressure response curve (DR - 1) at different times after administration of '511 100 mg, '511 200 mg, and ramipril 10 mg for 4 days

After dosing on Day 7, there was a reduction in median ACE activity from baseline irrespective of treatment. Treatment with ramipril 10 mg showed a significantly greater reduction in ACE activity when compared with '511 100 mg or '511 200 mg. The ratio of trough ACE activity with '511 100mg compared with Ramipril 10mg was 4.94 (95% CI: 2.98-8.17) and that of '511 200mg compared with Ramipril 10mg was 4.14 (95% CI: 2.56-6.68).

The pattern of ACE activity followed a similar time course for each of the treatments, with the lowest ACE activity being achieved at approximately 3 hrs followed by a gradual return to baseline levels over the following 24 hrs.

#### Neutral endopeptidase (NEP) activity:

##### **Plasma ANP**

On Day 7, baseline median plasma ANP concentration was similar for the '511 100 mg and 200mg treatment groups (19.65 pmol/ml and 18.00 pmol/ml respectively) and higher than in the ramipril 10 mg group (10.45 pmol/ml).

After dosing on Day 7, '511 led to a dose dependent increase in plasma ANP concentration consistent with systemic NEP activity. The rise in median plasma ANP concentration following treatment with '511 100 mg and '511 200 mg was 23.40 and 26.20 pmol/ml respectively.

The pattern of plasma ANP concentration followed a similar time course following treatment with '511 100 mg and '511 200 mg with a peak of median plasma ANP concentration being achieved at approximately 16 hours. This was followed by a gradual return to baseline levels over the next 24 hours.

##### **Plasma cGMP**

There was no notable change from baseline median plasma cGMP concentrations from Day 1 to Day 7 for all three treatment groups.

On Day 7, baseline median plasma cGMP concentrations were similar for the '511 100 mg and 200mg treatment groups, 12.9 pmol/ml and 12.50 pmol/ml, respectively, slightly higher than in the ramipril 10 mg group, (9.80 pmol/ml).

After dosing on Day 7, there was a rise in median plasma cGMP concentrations irrespective of treatment (approximately 2 hr), although the change in plasma cGMP concentrations was significantly lower in those subjects treated with ramipril 10 mg, followed by a fall in median plasma cGMP concentration by 3 hr. The pattern of median plasma cGMP concentrations followed a similar time course for each of the treatments gradually return to baseline levels over the next 24 h.

## **Discussion**

NEP plays a role in the breakdown of several endogenous vasodilatory natriuretic peptides. Blockade of NEP potentiates the action of these peptides by inhibiting their breakdown. Thus far, the effect of NEP inhibitors on blood pressure has been modest and none of the candidate NEP inhibitors have been approved for clinical use. It has been proposed that the additional ACE inhibition offered by dual ACE/NEP inhibitors has the potential to be more effective in hypertension and possibly heart failure by blocking the formation of the vasoconstrictor, angiotensin II and the breakdown of vasodilatory natriuretic peptides.

The aim of this study was to further characterise the NEP activity of '511 a new dual inhibitor *in vivo* and to compare the potential of '511 to cause plasma ACE inhibition with that of a clinically relevant comparator, ramipril.

I was able to demonstrate that '511 significantly and dose dependently inhibits NEP. ANP concentrations were significantly higher after dosing with '511 100 mg and '511 200 mg when compared to ramipril 10 mg. In fact even the lower dose of '511 caused an increase in plasma ANP concentration comparable to that seen with candoxatril in healthy volunteers. Plasma levels of ANP more than doubled in response to treatment. (Northridge et al 1989, Jardine et al 1990).

A comparison with candoxatril is perhaps particularly relevant since it would appear the *in vivo* ACE inhibitory properties of '511 are limited. In support of previous *in vitro* demonstration of ACE inhibition by '511, I was able to demonstrate some dose dependent reduction in ACE activity. The comparison with ramipril however was disappointing. I demonstrated that ramipril has more than four times the inhibition of ACE activity and when compared with ramipril 10 mg there was very little reduction in the hypertensive response to angiotensin I infusion with either dose of '511.

Johnson et al have more recently studied the same doses of '511 but without active control in 123 patients with mild to moderate hypertension. They demonstrate a significant reduction in blood pressure associated with significant inhibition of ACE activity and an increase in serum ANP and urinary cGMP excretion. Interestingly plasma ANP changes resulting from NEP inhibition were the more powerful predictors of change in blood pressure when compared to serum ACE activity. Indeed the authors conclude that NEP and not ACE inhibition is important for the antihypertensive efficacy of '511 (Johnson et al 2006).

In conclusion, I believe that an agent that is both a NEP inhibitor, augmenting circulating natriuretic peptide production and an ACE inhibitor, reducing angiotensin II production, is an attractive therapeutic option in a range of cardiovascular diseases. However '511 offers only limited ACE inhibition and most importantly in my experience poor clinical effect when compared to an established pure ACE inhibitor. The experience with '511 may be useful in the development of other dual NEP/ACE inhibitors.

## **9 Neurohumoral effects of the new orally active renin inhibitor, aliskiren, in chronic heart failure**

### **Background**

The success of ACE inhibitors as a treatment for CHF (CONSENSUS 1987, SOLVD 1991) has encouraged the development of alternative approaches to the blockade of the RAAS (Pitt et al 1995, 1997, 2000).

In part, this is because ACE inhibitors also block the breakdown of bradykinin. Accumulation of bradykinin appears to cause certain adverse effects (e.g. cough and angio-oedema) which lead to ACE inhibitor intolerance in a proportion of patients.(Pitt et al 1995, Fuller and Choudry 1987, Kjekshus and Swedberg 1988, Kostis et al 1994, 1996) A more specific inhibitor of the RAAS should avoid these problems (although accumulation of bradykinin could also have benefits by promoting vasodilatation and fibrinolysis (Pitt et al 1995, 1997, 2000).

ACE inhibition also results in accumulation of angiotensin I, which may competitively overcome enzyme inhibition and lead to "RAAS escape" (Pitt et al 1995). Other enzymes, such as chymase, may also continue to generate angiotensin II (Urata et al 1990, Petrie et al 2001, McDonald et al 2001).

These and other considerations have led to the introduction of specific angiotensin II type 1 receptor antagonists (Pitt et al 1995, 1997, 2000). AIIRAs, however, lead to accumulation of angiotensin II and the consequences of this, through the actions of angiotensin II at other receptors, are unknown (Gottlieb et al 1993, Cao et al 1999).

Both ACE inhibitors and angiotensin II receptor antagonists interrupt the normal feedback suppression of renin from the kidneys; the rise therefore in renin leads to greater generation of angiotensin I and in turn angiotensin II.

An alternative approach to blockade of the RAAS is inhibition of renin, the rate limiting enzyme for the formation of angiotensin II (Fisher and Hollenberg 2001, Nussberger et al 2002). Until recently, the introduction of renin inhibitors into clinical practice was limited by low oral bio-availability, poor efficacy, short duration of action and high cost of chemical synthesis (Fisher and Hollenberg 2001, Nussberger et al 2002). These

problems have in part been overcome with the development of the new, orally active, non-peptide, specific renin inhibitor, aliskiren (SPP 100) (Nussberger et al 2002). Aliskiren offers a longer duration of action and improved efficacy when compared to previous inhibitors but continues to have a poor bioavailability at only 2.5%. A high-fat meal decreases the mean area under the curve (AUC) and peak concentration (C<sub>max</sub>) of aliskiren by at least 70%. Approximately 25% of absorbed aliskiren is excreted in the urine unchanged.

This study describes the first administration of this agent to patients with CHF.

## **Methods**

The primary scientific aims of this study were to compare the neurohumoral actions of aliskiren to (i) placebo, because aliskiren has never been given to patients with CHF before and also (ii) to compare the actions of aliskiren to those of an effective dose of an ACE inhibitor, because comparable or greater effects than an ACE inhibitor are desirable. It is, however, practically and ethically difficult to design a comparison with placebo. This is because it is both hard to identify patients not started on treatment with an ACE inhibitor therapy and undesirable to withdraw established ACE inhibitor treatment. Consequently, I adopted the study design in figure 9.1, involving only a short period (up to two weeks) of ACE inhibitor withdrawal. After a 5-7 day run-in period, patients were randomised to placebo (ACE inhibitor withdrawal), ramipril 5mg once daily or aliskiren 75 mg once daily for one week. After this week, patients taking placebo switched to one or other of the active therapies for the remainder of the study (a further 5 weeks, figure 9.1).

Study assessments. The primary assessments in this study were i) change in plasma renin activity and concentration of angiotensin II and aldosterone concentration and BNP from baseline to the end of one week of treatment (visit 3) [this involved a comparison of placebo, ramipril and aliskiren] and ii) change in these neurohumoral measures from baseline (visit 2) over visits 4-6 (i.e. during a further 5 weeks of therapy). Other assessments included clinical examination (body weight, heart rate and sitting and standing blood pressure) and routine biochemical and haematological measurements, made at baseline and varying intervals during the 6 weeks of randomised therapy, as indicated in figure 9.1.



Neurohumoral assays. Blood samples were collected for measurement of plasma BNP, angiotensin II and aldosterone concentrations. Plasma renin activity was also measured. Samples were handled, stored and analysed as described in chapter 2.

Data analysis and presentation. Comparisons between the change in the neurohumoral measurements from the pre-treatment visit (visit 2) and those made after one week of therapy (placebo, ramipril or aliskiren, visit 3) were made using a Wilcoxon matched pairs test and the comparisons between visit 2 and visits 4-6 were made using one way analysis of variance followed by Bonferroni's test for multiple comparisons.

## **Results**

Patient details: The details of the patients studied are given in table 9.1. 27 patients (3 female) were randomised. All had been taking an ACE inhibitor prior to randomisation. Two patients were withdrawn early from randomised therapy, because of worsening heart failure, one after 15 days and the other after 35 days.

### **PLACEBO V. ALISKIREN V. RAMIPRIL**

One week, three way treatment comparison.

Plasma renin activity (table 9.2): Plasma renin activity did not change with placebo. Even within one week of therapy, plasma renin was suppressed by aliskiren. As expected, plasma renin activity tended to increase with ramipril.

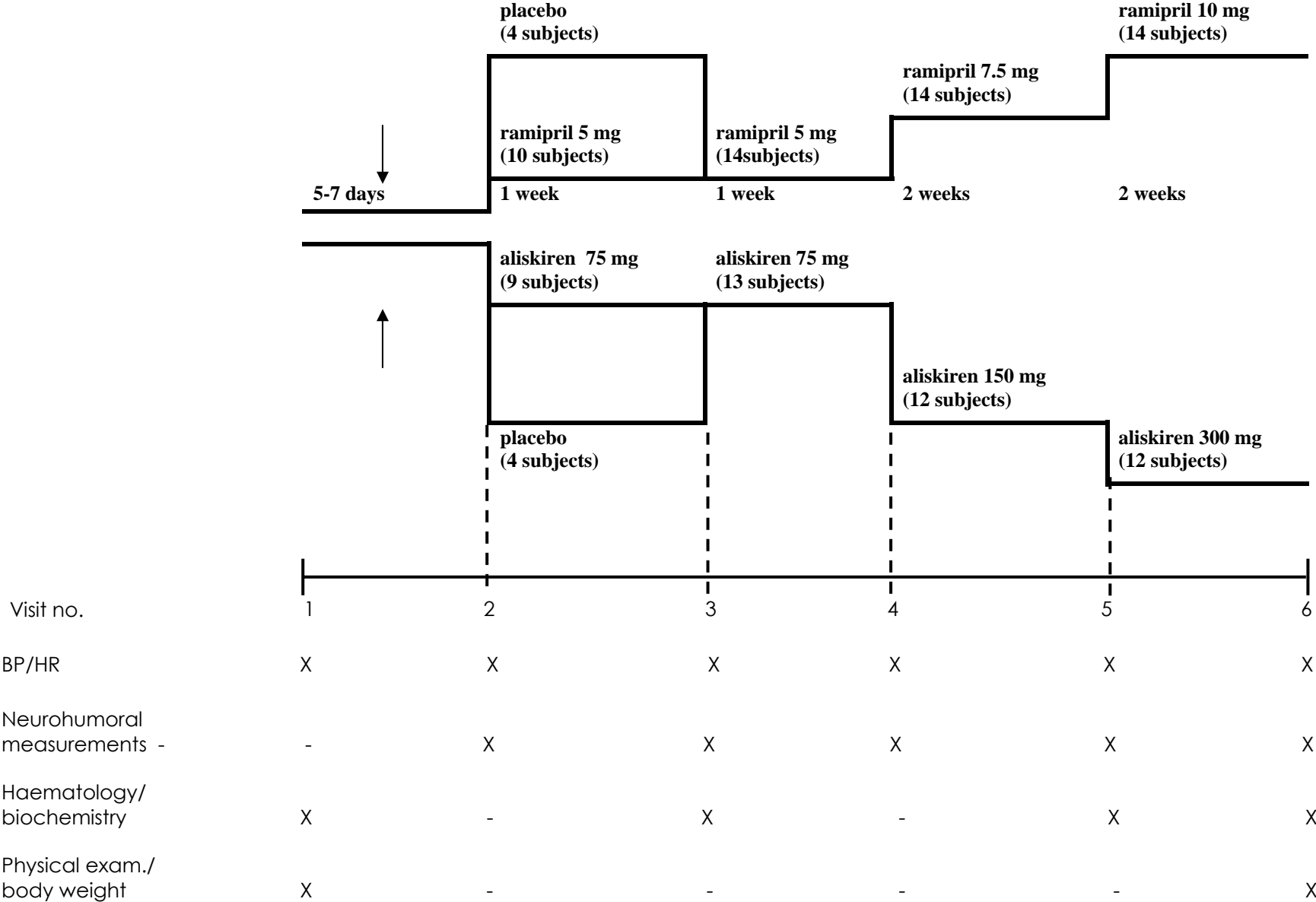
Plasma angiotensin II concentration (table 9.2): The plasma concentrations of angiotensin II did not change with placebo or ramipril. Angiotensin II, however, was clearly reduced by aliskiren.

Plasma aldosterone concentration (table 9.2): Plasma aldosterone concentration did not change significantly with any of the three treatments.

Plasma BNP concentration (table 9.2): Plasma BNP also did not clearly change with any of the 3 treatments.

Blood pressure (table 9.3): There were no significant changes in blood pressure in any of the three groups after one week of treatment.

**Figure 9.1** Study design and assessments



**Table 9.1** Baseline patient characteristics

Patient characteristics <sup>*</sup>	Initial treatment allocation		
	Ramipril	Aliskiren	Placebo
Number of patients	10	9	8
Age, years (SD)	66 (8)	65 (9)	60 (9)
Left ventricular ejection fraction	0.25	0.26	0.25
Drug therapy			
Beta-blocker	10	8	7
Spironolactone	0	1	0
Diuretic	7	7	6
Digoxin	5	3	2
HMG-CoA reductase inhibitor	5	7	3
Oral nitrate	4	1	0

<sup>\*</sup> number of patients, unless otherwise indicated.

HMG CoA, 3-hydroxy-3-methylglutaryl coenzyme A

## **ALISKIREN V. RAMIPRIL**

Five week, two way treatment comparison.

Baseline week 2 (follows 1 week drug free for all patients) compared to treatment weeks 4, 5 and 6. Week 3 is not reported since the only change at this timepoint was in the 4 patients who had continued on placebo for an extra week commencing treatment for the remaining 5 weeks.

Plasma renin activity (figure 9.2): As expected, aliskiren significantly reduced plasma renin activity whereas this increased with ramipril.

Plasma angiotensin II concentration (figure 9.3): Aliskiren also reduced plasma angiotensin II concentration whereas plasma concentrations of this peptide did not fall during ramipril treatment.

Plasma aldosterone concentration (figure 9.4): There was no clear change in plasma aldosterone concentration in response to either treatment.

Plasma BNP concentration (figure 9.5): There was no clear change in plasma BNP concentration in response to either treatment.

Blood pressure (table 9.3): There were comparable reductions from baseline in systolic and diastolic blood pressure with both treatments.

### Safety and tolerability

Aliskiren and ramipril treatments were generally well tolerated. A total of 24 adverse events were reported in 9 patients during aliskiren treatment and 15 adverse events were reported in 9 patients during ramipril treatment. Dyspnoea (4 patients) and fatigue and dizziness (3 patients) were the most frequently reported adverse events. The frequency of adverse events did not increase with the dose of aliskiren or ramipril.

**Table 9.2** Three way comparison of effect of one week of treatment with placebo, ramipril or aliskiren on plasma neurohumoral concentrations in patients with chronic heart failure

	<b>PRA</b> <b><u>ng/ml/hr</u></b>			<b>Angiotensin II</b> <b><u>pg/ml</u></b>			<b>Aldosterone</b> <b><u>ng/100ml</u></b>			<b>BNP</b> <b><u>pg/ml</u></b>		
	<u>visit 2</u>	<u>visit 3</u>	p	<u>visit 2</u>	<u>visit 3</u>	p	<u>visit 2</u>	<u>visit 3</u>	p	<u>visit 2</u>	<u>visit 3</u>	p
placebo	3.1(5.1)	2.4(4.1)	ns	49(67)	41(63)	ns	18(13)	14(9)	ns	248(179)	202(316)	ns
ramipril	0.9(0.8)	2.3(2.8)	0.03	10(9)	13(26)	ns	13(8)	12(10)	ns	224(326)	202(316)	ns
aliskiren	1.6(1.5)	0.4(0.3)	0.01	20(17)	8(8)	0.01	14(7)	11(6)	ns	126(102)	145(139)	ns

Visit 2 = pre-treatment; visit 3 = one week post-treatment (end of the three way treatment comparison period)

All values are means ( $\pm$  standard deviation)

PRA = plasma renin activity

BNP = B-type natriuretic peptide

**Table 9.3**

Three way comparison of effect of one week of treatment with placebo, ramipril or aliskiren on systolic and diastolic blood pressure in patients with chronic heart failure

	SBP (mmHg) ‡			DBP (mmHg) ‡		
Initial treatment	V2	V3	V6	V2	V3	V6
placebo (n=8)	133(11)	134(18)	-	75(6)	79(8)	-
ramipril (n=10)*	130 (24)	127 (22)	120 (21)	74 (14)	74 (15)	72 (13)
aliskiren (n=9)*	139 (10)	133 (18)	130 (21)	78 (14)	74 (8)	73 (11)

SBP = systolic blood pressure

DBP = diastolic blood pressure

V2 = visit 2 (pre-treatment)

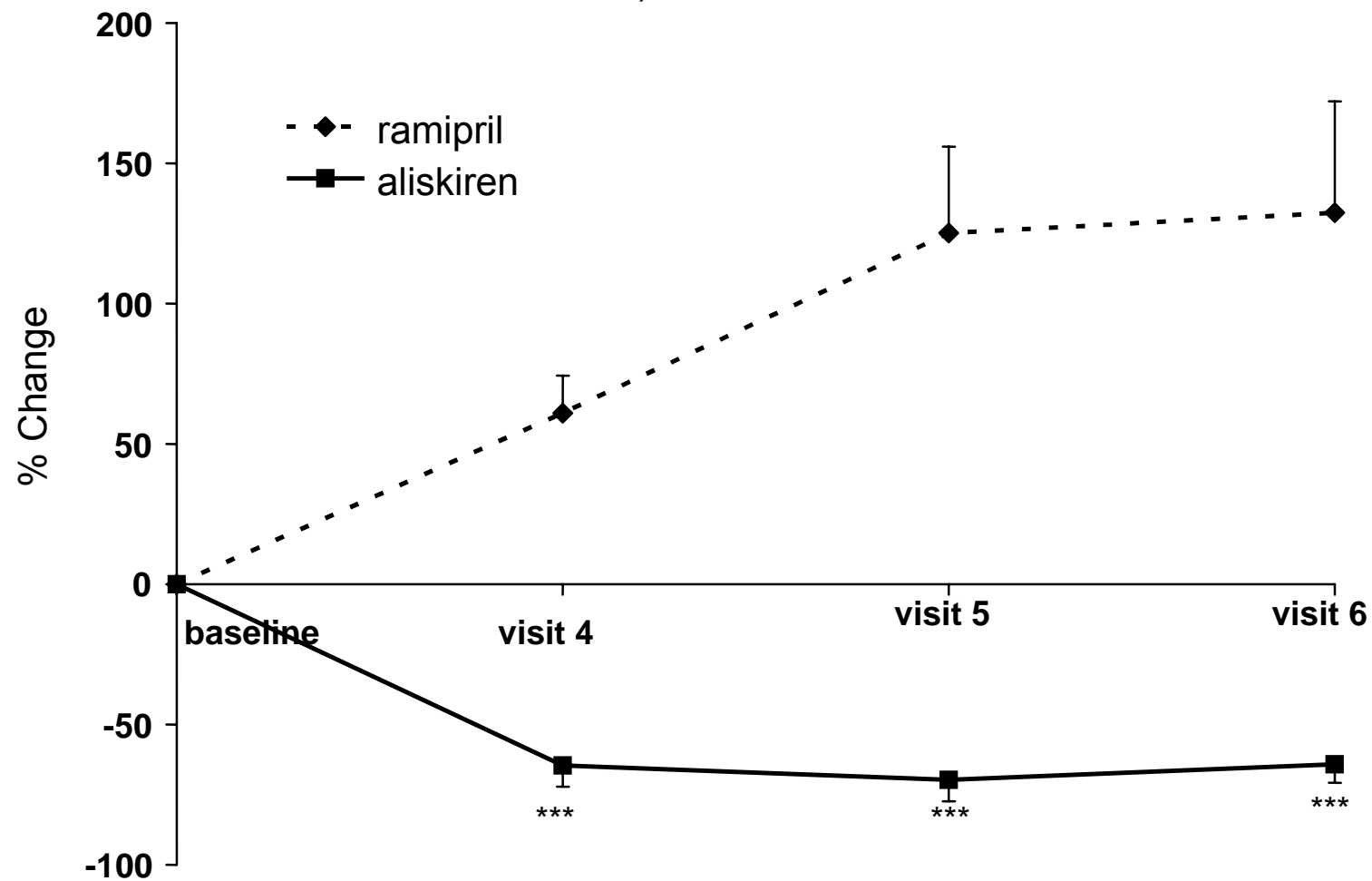
V3 = visit 3 (1 week's treatment)

V6 = visit 6 (6 weeks treatment); placebo was given for 1 week only.

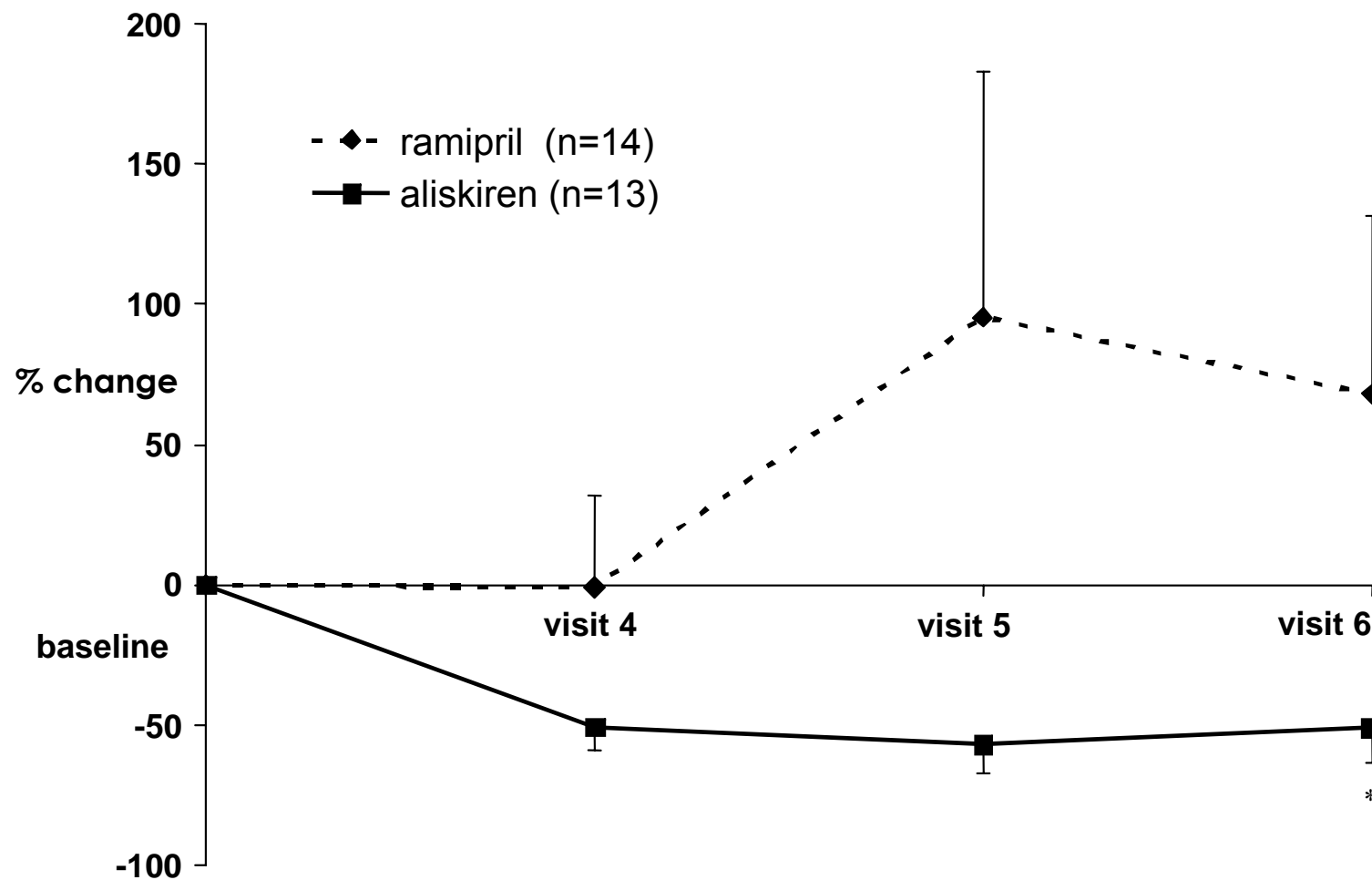
\* data for patients starting with ramipril/aliskiren only; patients switching placebo to ramipril/aliskiren after 1 week are not included.

‡ data presented as mean (standard deviation)

**Figure 9.2** Effect of aliskiren and ramipril on plasma renin activity (mean percentage change from visit 2 / baseline) \*\*\*  $p < 0.0001$

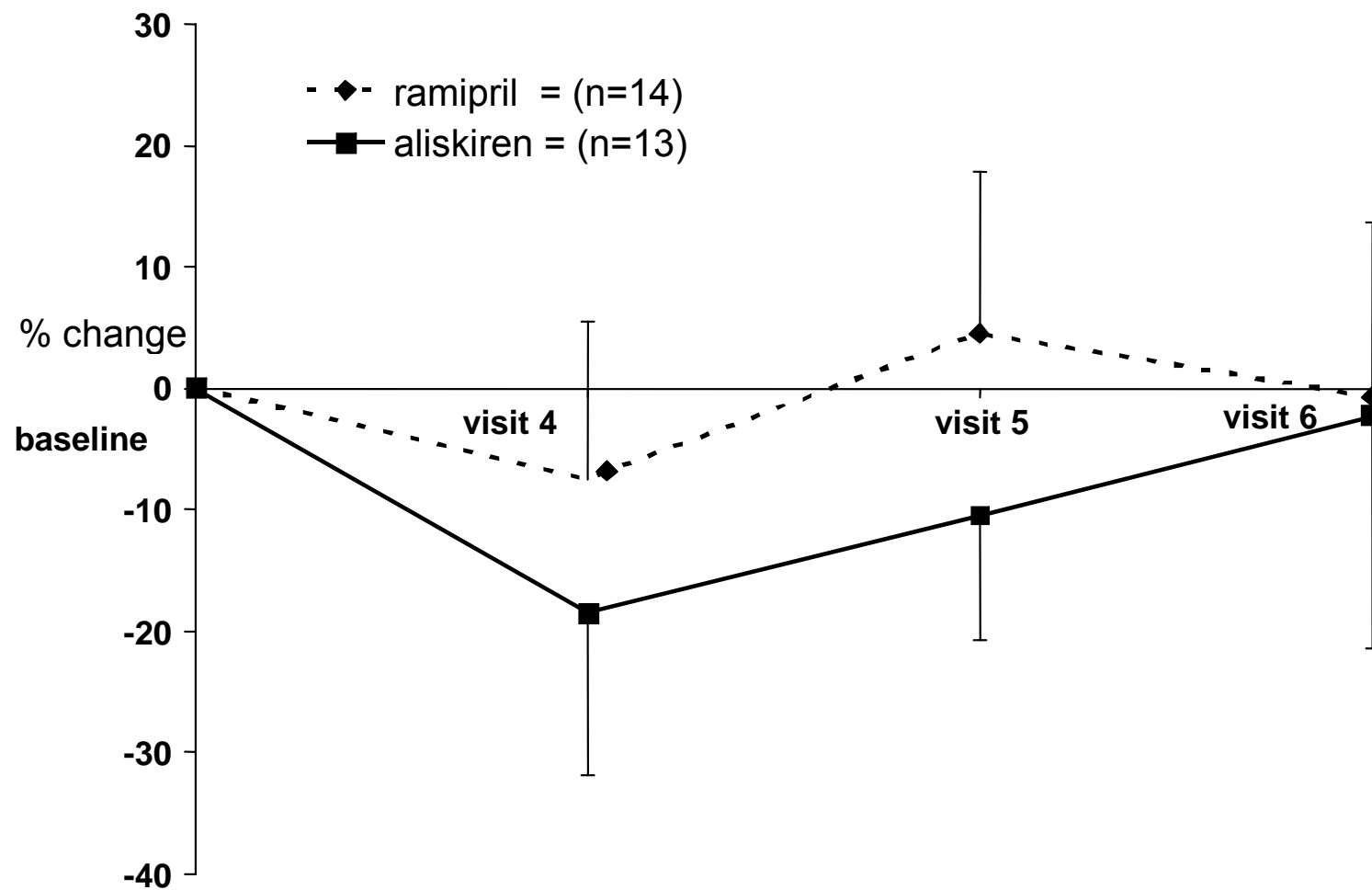


**Figure 9.3** Mean percentage change in plasma concentration of angiotensin II from visit 2 (baseline) \*  $p < 0.05$

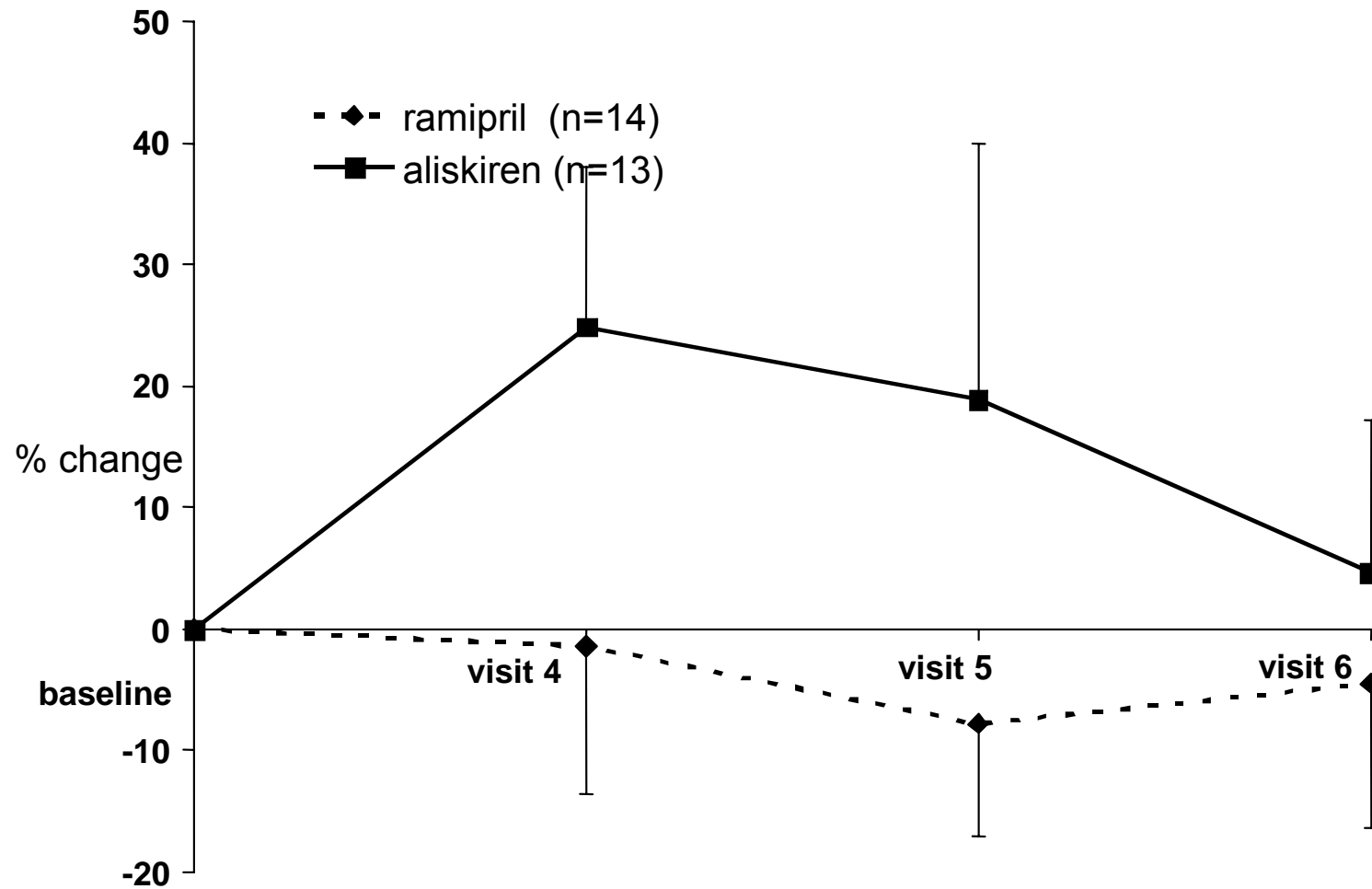




**Figure 9.4** Mean percentage change in plasma concentration of aldosterone from visit 2 (baseline). No difference between treatments was detected



**Figure 9.5** Mean percentage change in plasma concentration of BNP from visit 2 (baseline). No difference between treatments was detected.



## Discussion

Though a number of renin inhibitors have been studied in healthy volunteers and in patients with hypertension, there is very little prior experience with these agents in CHF (Delabays et al 1989, Boger et al 1990, Neuberg et al 1991, MacFadyen et al 1995, Van den Meiracker et al 1999, Staessen et al 2006).

The short-term haemodynamic effects of the selective dipeptide inhibitor enalkiren (A-64662), administered intravenously, were reported over a decade ago (Neuberg et al 1991). In that uncontrolled study, enalkiren resulted in favourable changes in cardiac index, systemic and pulmonary vascular resistance, left ventricular filling pressure and other indices of cardiac function. I know of no other studies with renin inhibitors in CHF (Neuberg et al 1991). In particular, I know of no chronic dosing studies with an orally active renin inhibitor prior to my work.

Aliskiren is an orally active renin inhibitor with a long duration of action. It has been studied in healthy volunteers (Nussberger et al 2002); doses of 40 to 640 mg daily were compared to 20 mg enalapril daily or placebo, with each drug given for eight days. Aliskiren was well tolerated and dose-dependently inhibited the RAAS to at least the same extent as enalapril (Nussberger et al 2002). Indeed, in healthy subjects, 160mg aliskiren gave equivalent suppression of angiotensin II as 20mg of enalapril. More recently, the efficacy of aliskiren (37.5mg, 75mg, 150mg and 300mg once daily for four weeks) has also been compared to losartan (100mg once daily) in patients with essential hypertension. In that study, 75mg and 150mg of aliskiren reduced blood pressure and 300mg lowered blood pressure to the same extent as losartan 100 mg (Stanton et al 2003). Subsequent studies have confirmed the effectiveness of aliskiren as an antihypertensive agent and compared it to other types of blood pressure lowering therapies (Gradman et al 2005, O'Brien et al 2007, Pool et al 2007, Jordan et al 2007, Oh et al 2007). The most recently published study looks at treatment of hypertension with aliskiren and with a combination of aliskiren and an angiotensin II receptor antagonist, valsartan. While again reporting significant reduction in blood pressure with aliskiren, -12.6mmHg systolic and -8.1mmHg diastolic, the combination provided only modest additional hypotensive effect, -4.4 mmHg systolic and -3.2 mmHg diastolic (Opavil et al 2007). Concern has been expressed regarding the potential for life threatening adverse event with the combination, particularly as a result of

hyperkalaemia, hardly balanced by this modest benefit (Birkenhager and Staessen 2007).

I have now studied Aliskiren in CHF. At the doses used in my study, the orally active renin inhibitor, aliskiren was at least as effective at suppressing the RAAS as the ACE inhibitor ramipril over a six week period. This was apparent acutely, where one week of treatment with 75 mg of aliskiren reduced plasma angiotensin II concentrations, in contrast to 5 mg of ramipril. This effect was also apparent over the subsequent five weeks of treatment where 75–300 mg of aliskiren more clearly reduced angiotensin II than ramipril 5-10 mg.

In the first study in hypertension mentioned above, angiotensin II was not measured but plasma renin activity was reduced from baseline by 55% with 37.5mg of aliskiren, by 60% with 75mg, by 77% with 150mg and by 83% with 300mg (contrasting with a 110% rise with losartan, Stanton et al 2003). I found a comparable reduction (-64%) in plasma renin activity with aliskiren in patients with chronic heart failure (compared with a 132% increase with ramipril) in the present study.

Why might renin inhibitors be valuable in CHF? Renin is the rate limiting step in the RAAS cascade and has a very specific interaction with its substrate. Consequently, adverse effects should be uncommon with renin inhibitors. Secondly, renin inhibition is the only approach to RAAS suppression that reduces both angiotensin I and II. This has several potentially favourable consequences. The reflex increase in angiotensin I which occurs during treatment with ACE inhibitors and angiotensin receptor antagonists does not occur during renin inhibition. This increase may ultimately overcome ACE blockade and lead to ‘renin-angiotensin-aldosterone escape’ (Pitt et al 1995). We know angiotensin I is also a substrate for other enzymes (eg chymase) capable of generating angiotensin II. Similarly, the excess angiotensin I produced during ACE inhibition (and the excess angiotensin I and II produced during treatment with an angiotensin receptor antagonist) may be converted to other angiotensin peptides which may directly and indirectly, produce both desirable and undesirable effects (Stegbauer et al 2003, Cesari et al 2002). Similarly the pros and cons of the bradykinin enhancing effects of ACE inhibitors (and the stimulating effect of angiotensin II on other angiotensin receptors during treatment with an angiotensin receptor antagonist) have already been alluded to. Overall the pharmacological consequences of each separate approach to inhibiting the

RAAS are myriad and quite different. Whether the different net pharmacological effect of each approach results in a different therapeutic action is unknown and difficult to predict. However, given the clinical success of the established pharmacological approaches to RAAS inhibition in cardiovascular and other diseases, it must be of interest to examine the clinical effects of renin inhibition, especially in CHF.

As with any study of this type, there are limitations. A relatively small number of patients were randomized and treatment was administered for a comparatively short period. Necessarily, my patients were those tolerating chronic ACE inhibition and the study was really one of ACE inhibitor withdrawal (and placebo or aliskiren substitution). This may explain the somewhat curious observation that angiotensin II tended to increase over time in patients randomized to ramipril. The present study was not a dose response study and investigated only one target dose of aliskiren. This was because 300mg had already been identified as an effective and well tolerated dose in the first hypertension study described above (Stanton et al 2003). Though there were no safety concerns with aliskiren in the current study, the number of patients studied and the duration of treatment were too limited to draw any firm conclusion.

In summary, this study shows that once daily dosing with aliskiren suppresses the RAAS as effectively as ramipril in the short term. This new approach to neurohumoral modulation is theoretically therapeutically attractive, especially in CHF, given the clinical success of the established pharmacological methods of suppressing the RAAS in this syndrome. Further studies of renin inhibition in CHF are clearly justified, indeed preliminary results of a prospective, multicentre, randomized, double blind study – ALOFT have recently been presented to the European Society of Cardiology.

## 10 DISCUSSION

The primary objective of this thesis was to characterise two recently developed molecules that have dual enzyme inhibitor activity, omapatrilat and SLV 306. Both are expected to augment the potent vasodilator natriuretic peptides via their effect on NEP. Both also inhibit potent vasoconstrictors, either angiotensin II or ET-1, via effect on ACE and ECE respectively. The combination in both instances promises significant therapeutic benefit in hypertension, CHF and even atherosclerosis and significant reduction in blood pressure in a hypertensive population has already been clearly demonstrated in the case of omapatrilat.

I have demonstrated that both molecules act on multiple vasoactive mediators and I am therefore able to offer possible explanation for, as well as further demonstration of, their clinical characteristics.

Omapatrilat is the most clinically advanced of the vasopeptidase inhibitors. It is orally active and offered great therapeutic potential in patients with hypertension or chronic heart failure. As an inhibitor of NEP in addition to ACE the challenge for this molecule was to achieve additional benefit over that achieved by ACE inhibition alone. It was hoped that the potential of NEP inhibition suggested by in vitro studies would finally be realised in vivo by the dual inhibitor. The explanation offered for failure to achieve this potential in the past with pure NEP inhibitors is that NEP not only has a role in the metabolism of the natriuretic peptides but also angiotensin II.

We are encouraged by large randomised controlled studies which report significant reduction of blood pressure by omapatrilat when compared to ACE inhibition alone, in hypertensive patients (Rouleau et al 2000, Kostis et al 2004). Unfortunately, the potential for clinical use in this population has been severely limited by the incidence of the potentially life threatening side effect, angioedema. This was not predicted following the earlier IMPRESS study. Perhaps explained by the fact that this was a selected population, already demonstrated to be ACE tolerant and the study was only over a short time period. We know that the occurrence of angioedema in patients taking ACE inhibitors can be as long as eight years following first prescription.

In the CHF population, a group with a poor prognosis and quality of life even on current therapy including an ACE inhibitor, a risk of serious adverse event may have been

acceptable to some. Unfortunately in an unselected heart failure population studies have suggested no significant benefit from vasopeptidase inhibition over and above ACE inhibition alone.

The profound effect of omapatrilat on systemic blood pressure, demonstrated in large randomised controlled trials and the unexpectedly high incidence of angioedema suggests that the inhibition of NEP and ACE has a more complex affect than simply natriuretic peptide augmentation and angiotensin II inhibition.

It is clearly important that we more fully understand the mechanism by which angioedema occurs as well as the relative contribution of inhibition and augmentation of various vasoactive hormones, to the benefits associated with this group of molecules.

CGRP and ADM are structurally similar peptides and bind to similar receptors. Both are powerful vasodilators and natriuretic peptides and CGRP particularly has been associated with dilatation of cutaneous arterioles and facial flushing, raising the suspicion that either or both of these molecules may play a role in the development of angioedema. Substance P and VIP are also powerful vasodilators with a suggested role in heart failure.

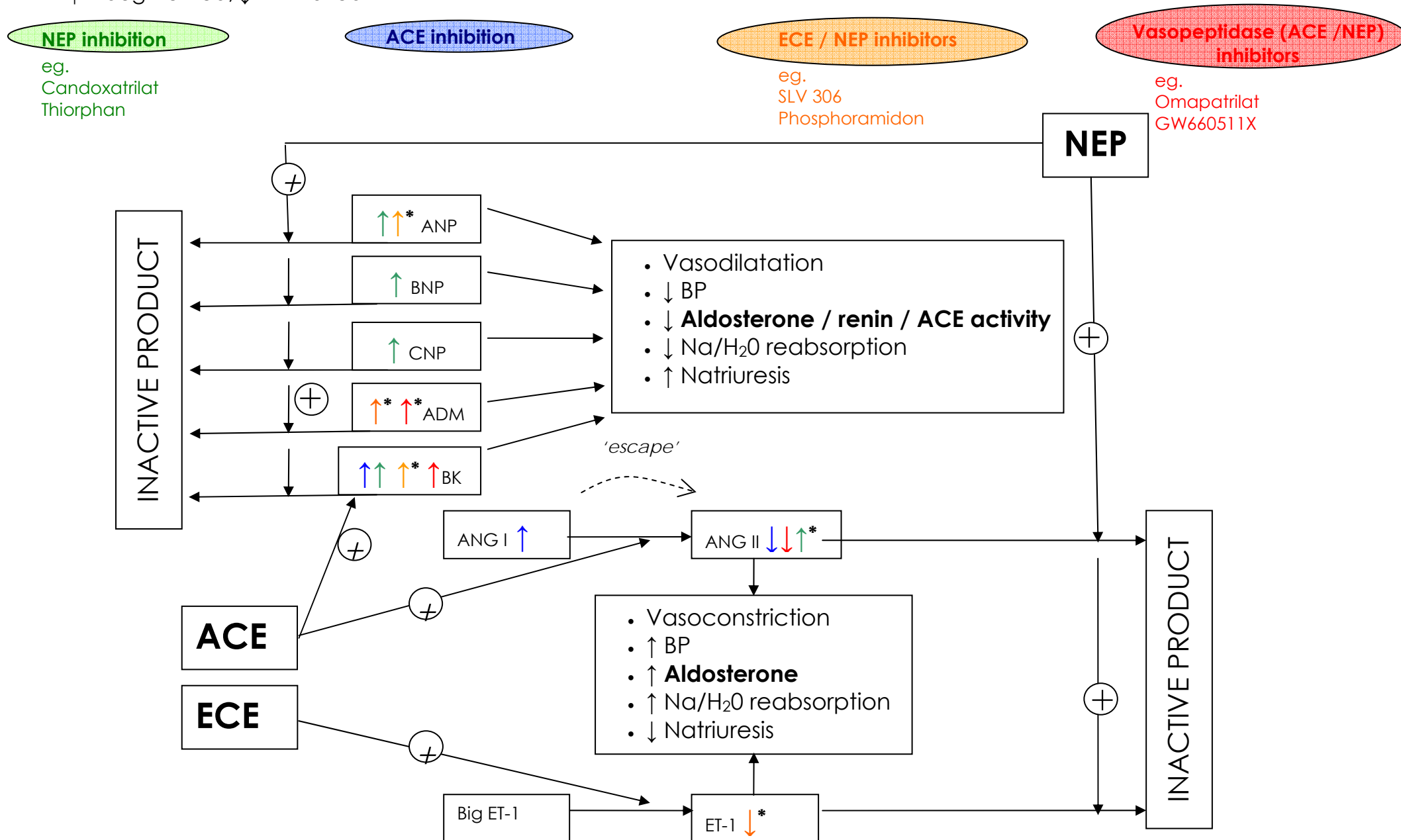
The effect of vasopeptidase inhibition on the action of these peptides in humans has not previously been studied. There has been the suggestion that NEP is responsible for the degradation of CGRP and ADM but the evidence is contradictory. There is evidence that VIP is augmented by ACE inhibition.

I was able to demonstrate that omapatrilat does indeed inhibit vasoconstriction in response to angiotensin I, consistent with ACE inhibition. Curiously however omapatrilat did not augment the vasodilatation response to CNP. I suggest that this reflects a difference between CNP metabolism and that of other natriuretic peptides rather than the absence of NEP activity with the dual inhibitor. Vasodilatation response to CNP has failed to be augmented in the presence of NEP inhibition previously (Brandt et al 1997). In retrospect it may have been more useful to study the effects of omapatrilat on the more consistent ANP or BNP than CNP in my studies.

**Figure 10.1**

The pathways affected by vasoactive enzyme inhibition including original contributions from this thesis \*

↑ = augmented, ↓ = inhibited





Of particular interest from my studies is the demonstration of omapatrilat's positive effect on the vasodilator response to ADM, raising the clear possibility that ADM contributes to the potent antihypertensive effects seen with the vasopeptidase inhibitor as well as perhaps the angiooedema known to be associated with it.

With no such effect on the response to CGRP, substance P and VIP I can suggest no similar role for these molecules.

NEP inhibition alone was not shown to augment the vasodilatory effects of ADM in a previous study in heart failure patients (Petrie 2001). My study population had a history of ischaemic heart disease but normal left ventricular function. A less potent effect of ADM in heart failure patients compared to those with normal left ventricular function has been demonstrated previously (Nakamura et al 1997).

We know ADM is released by endothelial cells and that a suggested mechanism of action is its effect on NOS and secondary augmentation of endothelium mediated vasodilatation. The action of ADM is dependent to a degree therefore on endothelial function. We know that patients with ischaemic heart disease have a degree of impairment in endothelial function. There are studies that describe acetylcholine causing vascular smooth muscle mediated vasoconstriction rather than endothelial mediated vasodilatation of coronary arteries in this population (Ludmer et al 1986, Gordon et al 1989). However, the subcutaneous vessels from ischaemic heart disease patients in my study were first tested for endothelial viability and showed a physiological vasoconstrictor response to acetylcholine. In the heart failure population, a lack of augmentation of ADM mediated vasodilatation in response to NEP inhibition may not represent a lack of ADM augmentation but a relatively poor endothelial cell response to it. In rat pulmonary arteries, the endothelium is necessary for ADM mediated relaxation (Yang et al 1996) Further studies looking at the response to NEP inhibition of ADM mediated vasodilatation in vessels with the endothelium removed may be useful. A difference in endothelial function and therefore vessel response to augmented ADM between patients with normal left ventricular function and patients with heart failure may explain the disappointing results seen in the large heart failure studies after such positive results were reported in the hypertensive population.

Augmentation of ADM and its actions, in response to NEP inhibition, has been demonstrated by other groups. Lisy et al (1998) however were not measuring vasomotor response to ADM but serum plasma levels following infusion and natriuresis and diuresis as a result of decreased sodium absorption. Rademaker et al (2002) studied the effect of NEP inhibition in the setting of heart failure but on the response to intravenous ADM. They considered plasma levels of ADM and renal function as well as systemic blood pressure and peripheral resistance. Although both groups demonstrated augmentation of the response to ADM in the presence of NEP inhibition this is likely to be explained by systemic as well as local reflexes, natriuretic peptide may in some way reduce ADM clearance (Bunton et al 2004). Most recently, augmentation of adrenomedullin by omapatrilat was also demonstrated by another group (Cataliotti et al 2002) in experimental heart failure although again at a systemic level. They demonstrated an increase in plasma levels of adrenomedullin following administration of omapatrilat.

These results do not directly contradict the findings of Petrie et al, whose findings are perhaps more a measure of endothelial viability in heart failure.

Perhaps more surprising from my studies is that while the dual inhibitor, omapatrilat, augmented the response to adrenomedullin, NEP inhibition alone was not associated with a more potent vasodilatation response than control. One suggested explanation is that the pure NEP inhibitor used, thiorphan, is a weaker NEP inhibitor than omapatrilat or the NEP inhibitors used in previous studies. However the effect of omapatrilat on ADM may also be more complex than NEP inhibition alone; angiotensin II has been associated with upregulation of ADM by an unknown mechanism (Pearson et al 2006). ACE inhibition may also play an important role in my findings. I do note however that captopril alone failed to augment ADM in my studies. It is possible to speculate that the augmented response to ADM at a local level is the result of the combined affect of ACE inhibition on endothelial function (particularly in ischaemic heart disease patients perhaps) and NEP inhibition on ADM degradation.

Supporting this more complex explanation for my findings is the fact that candoxatrilat, a powerful pure NEP inhibitor, shown to augment the systemic effects of ADM by Lisy et al, was not associated with angioedema in the hypertensive population in which it was widely studied.

Another strong candidate, according to the literature, for a role in the incidence of angioedema seen with ACE inhibitor therapy is bradykinin. It has been suggested that the higher incidence associated with omapatrilat may be the result of a dangerous synergy between ACE and NEP inhibition on bradykinin metabolism. While I demonstrated an increased response to bradykinin in the presence of omapatrilat, the fact that it was only similar and not greater to that seen with the ACE inhibitor, captopril counters this concern. It would seem that bradykinin is maximally augmented by ACE inhibition and that NEP inhibition does not lead to further augmentation. This is supported by my work with the dual inhibitor SLV 306. The NEP and ECE inhibition offered by this molecule was also unable to further augment bradykinin in the presence of ACE inhibition. Perhaps this finding could allay concerns about angioedema (Messerli and Nussberger 2000). Of course, I have only studied small resistance arteries and the ratio of NEP and ACE differs in other tissues e.g. the myocardium (Blais et al 2000). The effect of combined therapy with an ACE inhibitor and a dual NEP/ECE inhibitor could differ between tissues.

When considering both the additional hypotensive effect of omapatrilat and the increased incidence of angioedema when compared with ACE inhibition alone, the clear difference between dual inhibitor therapy and pure ACE inhibitor therapy on the response to ADM would suggest that this hormone rather than bradykinin is the more significant.

My findings support further work with other dual inhibitors. It is important that the results from studies of omapatrilat treatment and particularly the concerns regarding adverse events are not unreservedly extrapolated to newer molecules. Instead the encouraging results in a hypertensive population seen with omapatrilat and other vasopeptidase inhibitors should maintain enthusiasm for these potential therapies. A different balance of NEP and ACE inhibition may offer some but not all of the effects of ADM augmentation and a fear of more powerful bradykinin augmentation may not be valid. It is clear that development of other vasopeptidase inhibitors may still be of benefit.

My studies of another such agent '511 were unfortunately disappointing in that I demonstrated predominantly NEP inhibition. '511 does not apparently offer significant inhibition of ACE activity. While NEP inhibition alone is potentially beneficial, it is

limited in practice by the augmentation of vasoconstrictors as well as vasodilators as demonstrated by candoxatrilat.

Further work with this molecule has been reported more recently (Johnson et al 2006). A randomized double blind placebo controlled study in 123 patients with hypertension, using 100mg and 200mg of '511 as in my study, did demonstrate a significant reduction in systolic and diastolic blood pressure. Unlike my study an active control was not used but compared to placebo they did demonstrate a significant reduction in ACE activity as well as augmentation of ANP. Larger studies are obviously required. Of particular relevance is the fact that in Johnson's study, although relatively short and with small numbers of mainly caucasian subjects, there were no cases of angioedema. The authors suggest that this molecule increases bradykinin accumulation to a much lesser extent than previous vasopeptidase inhibitors such as omapatrilat, perhaps offering benefit therefore without significant side effects in their view.

The most advanced vasopeptidase inhibitor after omapatrilat, Ilepatril (AVE 7688), has been given to more than 1700 patients with hypertension in a recently closed study. This was a prospective, multicentre, randomized, double blind study with losartan as active control, designed to reflect clinical practice. As well as neurohumoral and haemodynamic data we await particularly the adverse events reported over the 52 weeks – a pre defined end point.

The concept of triple inhibitors has recently also gained interest. For example, ACE/NEP inhibition supplemented by additional inhibition of ECE. Preliminary studies, in experimental settings such as spontaneously hypertensive rats and rats with CHF, have shown that these molecules (Battistini et al 2005, Mellin et al 2005, Daull et al 2005) dose dependently reduce blood pressure, angiotensin II and ET-1, with an increase in big ET-1, ANP and bradykinin. Clearly careful evaluation of the safety profile of this strategy is needed before these molecules are considered as treatment options in humans.

We do not understand why some patients suffer angioedema with omapatrilat or ACE inhibitors and others do not. Studies have not reported a correlation between blood pressure response and risk of angioedema. It may be that the activity of one vasodilator when compared to another varies between patients. In some, ADM and bradykinin may

be the more active, putting the patient more at risk of side effects. In others the effect on natriuretic peptide activity may predominate and the contribution from the other vasodilators is enough to cause a reduction in vascular resistance without the unwanted adverse event. This argument supports a growing belief that bespoke medication on an individual patient basis is the way of the future (Evans and Relling 2004) but does not facilitate development of widely effective and safe vasopeptidase inhibitors.

SLV 306 is the first dual NEP/ ECE inhibitor available in oral form and the first to be given to healthy volunteers or patients. Other molecules are in development but have not yet been administered to humans (Emoto et al 2005, Trapani et al 2002) Again these molecules hope to offer augmentation of natriuretic peptides as a result of NEP inhibition, without the augmentation of vasoconstrictors, specifically ET-1, another vasoconstrictor said to be metabolised by NEP and therefore augmented by NEP inhibition. Their role is likely to be in addition to ACE inhibition since Angiotensin II is also metabolised by NEP. Any synergy between molecules such as SLV 306 and ACE inhibition may therefore be of concern.

I was able to demonstrate potent ECE inhibition by this molecule with attenuation of the vasoconstrictor response to big ET-1 in vitro and attenuation of the pressor response to infused big ET-1. SLV 306 also caused an increase in the plasma big ET-1: ET-1 ratio following infusion of big ET-1 in vivo, with no increase in ET-1 but a dose dependent increase in big ET-1. I was able to demonstrate NEP inhibition with augmentation of the vasodilator response to ANP in vitro and an increase in plasma levels of ANP in vivo.

Our experience now with omapatrilat in large studies of hypertensive and heart failure patients highlights the importance of studying the effects of molecules such as SLV 306 on multiple vasoactive hormones. SLV 306 in addition to ACE inhibitor therapy offers broad vasoactive enzyme inhibition and the neurohumoral effects are likely to be complex and whilst beneficial potentially also detrimental.

As expected, KC12615 augmented the vasodilator response to bradykinin. This is likely to be as a result of NEP inhibition in the most part, the pure NEP inhibitor thiorphan showed a similar response. Of particular interest is that SLV 306 does not offer additional bradykinin augmentation in the presence of ACE inhibition. Perhaps limiting its therapeutic potential but also allaying fears about the potential problems associated

with an increase in the activity of a hormone implicated in the common side effect, cough, associated with ACE inhibition and the poorly understood but potentially life threatening side effect, angioedema also seen with ACE inhibition but more frequently with omapatrilat.

KC12615 does also augment the vasodilator response to ADM. It has been suggested that this hormone offers cardiovascular benefit in hypertension, atherosclerosis and heart failure (Shimosawa et al 2002, Cataliotti et al 2002, Nagaya et al 2000, Jougasaki et al 1996, Nakayama et al 1999) but my work raises concern regarding the role of ADM in angioedema and emphasises the need for caution with any broad vasoactive enzyme inhibition of this kind.

We await with interest the results of two studies of SLV 306 recently closed to recruitment in patients with hypertension, particularly the incidence if any of angioedema in the study population. The first is a placebo controlled multicentre study giving patients one of three doses of SLV 306 (Daglutril) or amlodipine 5-10mg (study identifier NCT00160212, [www.clinicaltrials.gov](http://www.clinicaltrials.gov) accessed July 07). The second from a single centre adding SLV 306 (Daglutril) to losartan in hypertensive, diabetic patients with nephropathy (study identifier NCT00160225, [www.clinicaltrials.gov](http://www.clinicaltrials.gov) accessed July 2007).

Perhaps the principle motivation for the work reported in this thesis, is an appreciation of the complexity of the neurohumoral system in humans. Omapatrilat being a recent example of how unexpected the results can be when interfering with this system. Our understanding is apparently particularly limited when we act on more than one pathway.

Others also gain from the now broad experience with neurohumoral modulation in cardiovascular disease, utilizing therapies which modulate neurohumoral pathways in other systems.

ET receptor antagonists have been associated with outcome benefit in patients with pulmonary hypertension not unexpected since ET is particularly active in the pulmonary vasculature (Channick et al 2001). Inhibition of the endothelin system either by ET antagonists or ECE/NEP inhibition with molecules such as SLV 306 has been associated with a reduction in pulmonary as well as systemic pressures. The NEP and ECE inhibitory properties of SLV 306 having been studied in animal models of

pulmonary hypertension have shown a very promising profile of activity (Meil et al 1998).

Vasopeptidase (ACE / NEP) inhibition and ECE / NEP inhibition have both been associated with beneficial renal effects thought to be secondary to a combination of inhibition of RAAS, ET-1 production and NEP all associated with increased glomerular filtration rate and renal blood flow (Troughton et al 2000, McLean et al 2000, Meil et al 1998).

Vasoconstriction and secondary reduction in vascular compliance, increased cardiac afterload, pulmonary hypertension and renal disease is mediated in CHF and other cardiovascular pathologies at least in part by stimulation of the complex neurohumoral pathways that I have described. It makes sense that inhibition of these pathways by whatever means will offer benefit perhaps in the general heart failure population, perhaps in subgroups of heart failure patients particularly affected by common comorbidities such as pulmonary hypertension and renal disease, perhaps also in patients with only these associated co morbidities.

Renin inhibition, by acting at the origin of the RAAS perhaps offers patients much more specific neurohumoral modulation. The result being near to physiological is not associated with rises in undesirable reflex neurohormones such as angiotensin I and as a result is less likely to be associated with side effects. The pros and cons of complex neurohumoral modulation such as bradykinin or adrenomedullin augmentation have been well described. Whether there is also a difference in net pharmacological effect with each separate approach to inhibiting the RAAS or indeed other neurohumoral pathways remains unknown however and difficult to predict.

It is encouraging to see from my data that aliskiren is at least as effective at suppressing the RAAS as an ACE inhibitor in the heart failure population, as seen previously in the hypertensive population. In the hypertensive population trials have demonstrated an associated clinical benefit (Oh et al 2007, Oparil et al 2007) and recent results in the heart failure population are similarly encouraging.

Aliskiren has now been given to 302 patients with NYHA class II –IV heart failure, hypertension and a BNP concentration >100 pg/ml in a study recently reported to the European Society of Cardiology in Vienna. ALOFT, a prospective, multicentre,

randomized, double blind study was designed to reflect clinical practice. Patients had to be optimally treated with an ACE inhibitor or angiotensin II receptor antagonist and a  $\beta$  blocker, unless contraindicated or not tolerated. A group therefore in whom the compensatory rise in plasma renin, caused by loss of negative feedback inhibition of renin secretion, had occurred. They were studied for 3 months. Although primarily a safety and tolerability study, blood samples for neurohumoral analysis were taken. Compared to placebo, aliskiren reduced plasma BNP by 25%,  $p=0.0160$  and urinary aldosterone by 21%,  $p=0.0150$ . There was also a favourable change in a Doppler-echocardiographic measure of left ventricular filling pressure. Aliskiren was well tolerated and there was no significant excess of hypotension or renal dysfunction.

Taking note of concerns expressed previously (Birkenhager and Staessen 2007), with regard to the combination of renin inhibition and ACE inhibition or angiotensin II receptor blockade, it is important to note that even in this carefully selected population there was a slight increase in the incidence of hyperkalaemia in the aliskiren treatment group, although not reaching statistical significance. It is also important to emphasise that all of these patients had a history of hypertension as well as left ventricular impairment, with a slight difference in the mean blood pressure of treatment and placebo groups at baseline interpretation of the results is open to discussion although importantly there is no difference in blood pressure in either group between baseline and the end of the study.

In summary, favourable neurohumoral and other effects in otherwise optimally treated patients with heart failure were seen. This study, in addition to my own, supports further evaluation of aliskiren as a possible future treatment for heart failure. However, experience with other strategies, not least omapatrilat, highlights the need for only guarded optimism when considering the therapeutic potential of this class in the more complex heart failure population.

Of course ACE inhibition, now widely used in a broad spectrum of cardiovascular and related diseases, despite complex and not entirely understood effect on multiple neurohormones, is just one example of the potential gain from neurohumoral manipulation however and we should remain enthusiastic in our search for additional effective and safe therapies in this field.



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